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THE EFFECT OF OVER-ACTIVITY ON THE MORPHOLOGICAL STRUCTURE OF THE SYNAPSE

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FOURTEEN FIGURES

INTRODUCTION

Investigations on the histological manifestations of the nerve cell in fatigue have long been familiar to us Numerous authors have brought contributions to this important and interesting subject If we take for granted, as Sjoeval declared, that the alteration of nerve cell in tetanus is to be regarded as the effect of activity, the number of references becomes even more abundant It would suffice to point to those works of Hodge (14, 15 16), Vas (33), Mann (23), Lugaro (29), Sjoeval (32), Dolley (17, 18, 19), and many others There is almost a complete agreement on the point, that over-activity causes appreciable histological alterations in the nerve cell Kocher (19), who studied the same subject in our laboratory, could not, strangely enough, find any qualitative and quantitative changes in the histological characters between the fatigued and resting nerve cells Recently Saito undertook an exploration in the same line in this clinic, but the results are not published yet

Compared with the abundant researches on the neurocytological manifestations, there has been no attempt made to investigate the histological alteration of the synapse in fatigue, as far as I know Not only in regard to over-activity, but also in other pathological conditions, the histopathological changes of the synapse have been the topic of very few investigations And no wonder, for the structure of the synapse had not yet been conclusively demonstrated even in the normal condition, despite

many investigations by different authors (Golgi Bethe Held Perroncito Marinesco and many others) The lack of our knowledge concerning the pathological manifestations in the synapse and especially of experimental pathological data seems to be dependent on the difficulty of technique on the one hand and the impossibility of adequate material on the other In my recent publication [24] I described more minute structures of the synapse of the Mauthner cell of teleosts (bony fish) Perhaps I have not made the structure of the synapse clear beyond discussion however my results have been sufficiently definite for me to attempt experimental work on the pathological condition especially in fatigue Careful and thorough investigation on this topic led me to very interesting results which throw light on the histological condition of the synapse in functional activity although it was forced far beyond the physiological limit In the present paper I will describe mainly the manifestations in the synapse as it was not my purpose to investigate the neurocytological alterations of the Mauthner cell itself But wherever notable manifestations of the cell body appear they will be described as well as the findings of the synapse To Dr Adolf Meyer I beg to express my high appreciation here for his help and kind suggestions in this study

MATERIAL AND METHODS OF STUDY

In the present investigation Amiurus nebulosus was the material of experimentation Carassius auratus which offered me many interesting results in my previous work proved to be unfavorable material owing to the fact that it is weak and often dies before it comes to the utmost exhaustion It was always attempted in this experiment to work with strict control of the normal structure of the Mauthner cell as well as its synapse and care was always taken to avoid the formation of artefacts as much as possible

As a resting non-fatigued control which was provided in each experiment I used a fish of about the same size which was kept in a resting condition during the experiment of the other fish The body length of the fish used varied from 5 to 7 inches

The experiment consisted in forced activity of the fish, carried to the most advanced stage of fatigue, the experimental fish was placed in a jar 7 5 inches in diameter and about 8 5 inches in depth and then the water in the jar was continually stirred by running water, which came with high pressure from a faucet The fish swims in the stirred water, trying all the time to hold its equilibrium According to Bartelmez (3), the Mauthner cell participates in the equilibratory reflex, so it was supposed that in this experiment the Mauthner cell would be forced into continuous activity The duration of the experiment varied remarkably in each fish (from 24 to 98 hours) At first the fish swims actively and is able to hold its equilibrium very well, but gradually it gets tired and in the final stage of the experiment it is deprived of the ability of balancing so that it is moved passively tumbling around in the stirring water By this time if we stop the running water for a moment the fish would lie on its back or on one side, showing no attempt to maintain its upright posture As the sign of utmost exhaustion I chose a test, which consisted in holding the fish upside down by its tail in the water as well as in the air In the most advanced stage of exhaustion the fish did not flap at all even in this test

The fish was then decapitated and bled the brain was quickly but carefully dissected out and fixed in 10 per cent formalin, formol Zenker fluid and alcohol (95 per cent), respectively The resting control fish was killed at the same time and the brain was manipulated in quite the same way as the fatigued brain It must be emphasized also that the material was always fixed fresh and that material from fish which died was not examined

From the material thus obtained, the following preparations were made with the same technique as was described in my recent publication

1 Lhonim-cosin preparation (1 series each of normal and fatigued brains)

2 Toluidin blue preparation (5 series each of normal and fatigued brains)

3 Heidenhain preparation (6 series each of normal and fatigued brains)

4 Levaditi preparation (14 series each of normal and fatigued brains)

5 Bielschowsky preparation (6 series each of normal and fatigued brains)

6 Cajal preparation (5 series each of normal and fatigued brains)

Besides these I used the following stains in the present work

7 Scharlach stain (5 series each of normal and fatigued brain) The fish brain was fixed in 10 per cent formalin twenty-four hours, rinsed with water, cut at 15μ with the freezing microtome The sections were stained in saturated solution of Scharlach R in 70 per cent alcohol, rinsed with distilled water, counterstained with diluted Ehrlich's hematoxylin solution, washed again, and mounted in glycerin As there is only one pair of Mauthner cells in a fish brain, it was rather hard to get the sections in which the Mauthner cell is found

8 Mallory preparation (5 series each of normal and fatigued brain) In this preparation the brain was fixed first in 10 per cent formalin twenty-four hours and then placed in formol-Zenker fluid twenty-four hours Paraffin sections 5 to 8μ thick were stained with a diluted solution of Mallory's hematoxylin

9 S-fuchsin-light green stain (5 series each of normal and fatigued brains) Brains were first fixed in 10 per cent formalin twenty-four hours and then placed for eight days in the chromic acid acetic acid mixture Paraffin sections of 5μ were treated as follows 1) Remove paraffin from the sections and pass to 96 per cent alcohol 2) Place sections for one hour in a saturated aqueous solution of S-fuchsin in the incubator at 58°C 3) Wash twice with distilled water, till no more stain comes out of the sections 4) Dip the slides in motion 10 to 20 seconds in the following solution

Saturated alcoholic solution of picric acid	30
Aqua destillata	50

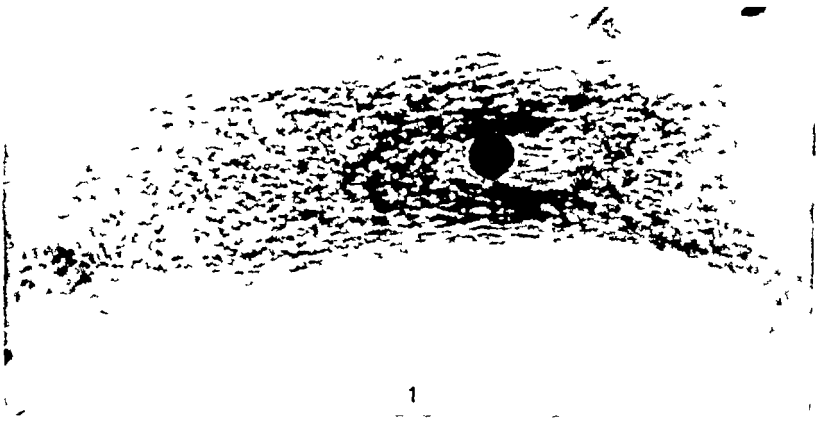
5) Rinse carefully twice in water 6) Place the slides in a saturated aqueous solution of light green twenty minutes The sections were then washed with water, dehydrated very quickly and passed into xylol

In all 122 series of normal and fatigued brains form the basis of the present article

ON THE INTERNAL MORPHOLOGY OF THE MAUTHNER CELL AND ON THE MINUTE STRUCTURE OF THE SYNAPSE

Under this heading I will make a few notes on the internal morphology of the Mauthner cell (figs 1 and 2) and on the structure of the synapse, which are necessary as the foundation of the following statement and were not yet described in my recent publication Nissl bodies are distributed evenly through the cell body and bases of the dendrites, leaving free only the axone hillock They are relatively small as compared with those in the motor cells and very numerous, as Bartelmez (3) stated The Nissl substance is found in the shape of variably long strine and is arranged generally parallel to the contour of the cell body and in part also to the surface of the nucleus The remaining stainable substance is irregularly scattered and is more or less short, some of it is spheroidal The spindles were found especially on the surface of the cell and in the larger dendrites The so-called nuclear caps did not come to my observation The axone hillock is entirely free from stainable substance and marked off by a tolerably sharp curved plane from the granular protoplasm of the cell body and shows at its margin a layer of especially fine granules The nucleus of the Mauthner cell differs in no essential from the typical nuclear structure of the nerve cell

As was precisely stated in my recent communication, the synapse of the Mauthner cell is penetrated by the Golgi network, which is formed by the arborization and the reunion of the delicate processes of the neuroglia cells in and about the 'axone cap' It was also accepted that the Golgi network is to be attributed to that category of the neuroglia tissue, which Held (24) termed as the reticular glia tissue formed by the somewhat modified plasma of the glia cell Furthermore, I paid special attention in that paper to the histological structure of the nervous elements of that synapse and the relation of the latter to the Golgi net We may therefore pass directly to the description of the finer structure of the glia cells themselves and the condition of the capillaries in this synapse



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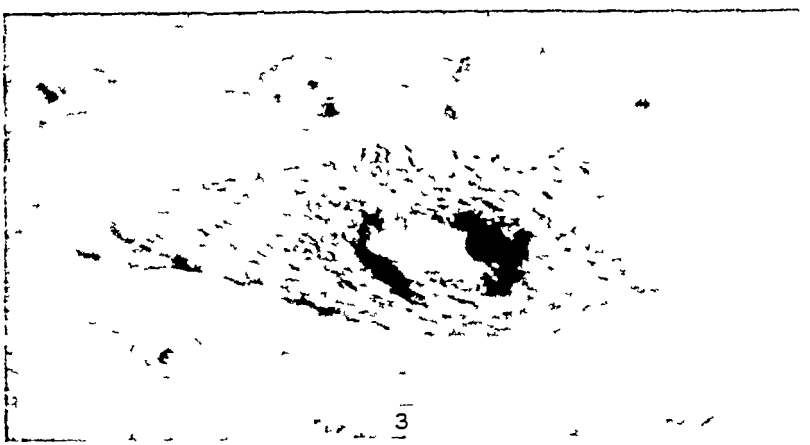

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The neuroglia nucleus is surrounded by a variously wide border of protoplasm, which latter sends protoplasmatic processes in different directions The process itself ramifies and becomes more and more delicate, until it passes into the beams of the Golgi net or becomes attached to the wall of a capillary This picture could very clearly be observed in the Levaditi preparations Heidenhain preparations of formalin material, Mallory and acid-fuchsin-light green preparations In the Heidenhain and thionin-eosin preparations of formol Zenker material a similar condition was demonstrated, although it was not so clear as in the above mentioned preparations

The protoplasm of glia cells was brought out favorably in the thionin eosin preparations, Heidenhain preparations Mallory and acid-fuchsin light green preparations There are two different types of neuroglia cells, in one type the protoplasm is very scanty so that the glia cell shows a small round cell body, while in another type we find a large mass of protoplasm around the nucleus The neuroglia nuclei are sometimes connected with each other not by means of the Golgi net substance, but by a variously broad mass of granular protoplasm I should interpret this as the result of amitosis which is observed now and then in the synapse It must be positively emphasized here, however that all the glia cells of *the synapse belong to one reticulum and that there is no cell individual* among them in normal brains The structure of the glia nucleus hardly calls for a description except that sometimes it shows evidences of amitosis, as Bartelmez (3) also stated Capillaries are found here and there in and about the synapse of the Muthner cell They do not offer anything particular in their

The figures 1 to 8 are the untouched photomicrographs taken from different preparations of both the control and the fatigued Amiurus brains In figure 2 and 3 an apochromatic Zeiss ocular no 4 and Zeiss objective D were used others were taken with the same ocular and a Zeiss immersion $\frac{1}{12}$ The length of bellows was 60 cm in all the photomicrographs The figures 9 to 11 were drawn from different preparations of fatigued Amiurus brains using the Abbe camera lucida (Zeiss apochromatic ocular no 4 Zeiss oil immersion $\frac{1}{12}$ tube length 20 cm)

Fig 1 Toluidin blue preparation (alcohol material) (control fish)

Fig 2 Thionin-eosin preparation (formol Zenker material) (control fish)

Fig 3 Toluidin blue preparation (alcohol material fatigued)

structure and consist of endothelium and adventitia with very few nuclei As already remarked, the adventitia of the capillary is connected with the bases of the reticular beams, but I was not able to distinguish the perivascular limiting membrane of Held as a membrane separated from the adventitia, so that I could not find the so-called perivascular lymph space between them in normal fish brains

THE HISTOLOGICAL MANIFESTATIONS OF THE MAUTHNER CELL IN FATIGUE

To recapitulate and discuss the results of other authors here would go beyond the purpose of the present work, my remarks will be restricted to my main results of investigation

The cell body of fatigued cells was found either in the state of turgescence (figs 3 and 4) or of shrinkage (fig 5), in the former case the cell border was convex between the dendrites and the dendrites appeared shorter, whereas in the latter the cell border was concave and the dendrites looked longer I agree with the opinion of Vas (33), Mann (23), Lugaro (21), Pugnat (26) and Holmgren (17), that the enlargement of the cell body is to be considered as the manifestation of activity and the shrinkage as that of exhaustion In this way the results of Hodge (14, 15, 16), which deviate from those of others, might well be interpreted Dolley (7, 9) described the fluctuations of the size of the cell body in the course of activity, but I have a little doubt about his statement that in later stages the absolute size of the cell body increases steadily to the end The alteration of the Nissl substance manifested itself in more or less advanced stages of chromatolysis, as was described by Vas (33), Lambert (20), Mann (23), Lugaro (21, 22), and many others, and thereby the cytoplasm was stained variously deeply (figs 3, 5) The Nissl bodies were found in a state of fragmentation, shortening, and irregular distribution, in the advanced stage of chromatolysis they were reduced to fine granules or even to a homogeneous substance Sometimes I observed the central beginning of the chromatolysis, as was stated by Vas (33), Mann (23), and, in tetanus, by Sjoeval (32)

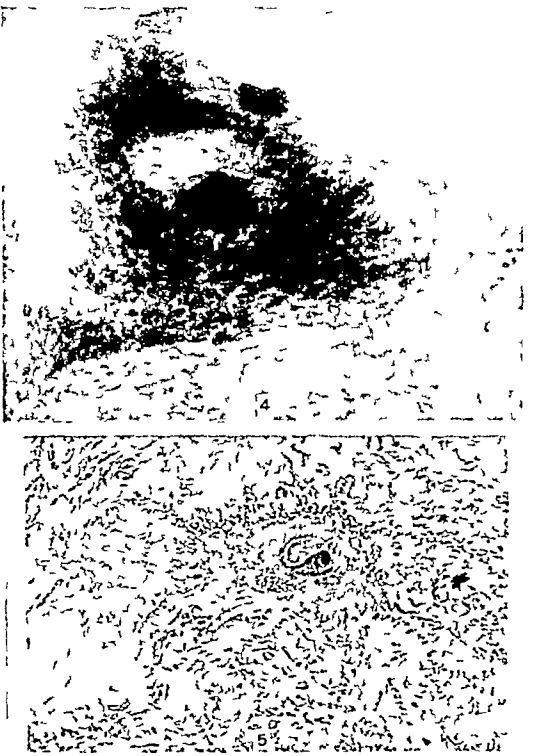
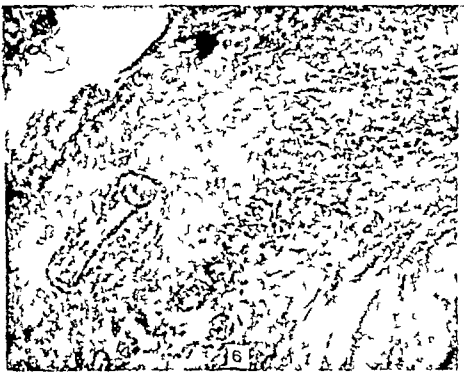


Fig. 4 Toluidin blue preparation (alcohol material) (fatigued)
Fig. 5 Phloxin-eosin preparation (formol Zenker material) (fatigued)

The nucleus was sometimes located in the center of the cell body but in many cases it was situated more or less eccentrically, as was described by many others—Vas (33), Lambert (20), Sjoeval (32), Holmgren (17) Morphologically, the nucleus showed now and then no particular change, but in other cases it was found either swollen or shrunken The swollen nucleus appeared large and round and showed a smooth nuclear membrane, whereas the shrunken nucleus showed irregular shape and a crenated membrane Vas (33) observed always an enlargement of the nucleus, while Hodge (14, 15, 15, 16) found regularly the shrinkage of the latter Lugaro (21), Mann (23), Pugnat (26), and Holmgren (17) declared on the basis of their study that activity causes turgescence followed by shrinkage in exhaustion Sjoeval (32) denied the pathological significance of this finding for the reason that he observed those nuclei also in the cell which showed no change in particular I think those manifestations of the nucleus are to be attributed to different stages of activity, Dolley (9) also declared that the nucleus shows fluctuations of size in the course of activity

The nucleolus was found mostly eccentric in the nucleus, although this was the case also in some resting nerve cells It was sometimes observed swollen (fig 4), in other cases it showed an irregular shape, oblong or angular (fig 5) Mann (23), Lugaro (21), Luxemburg (22) observed the enlargement of the nucleolus during activity, it disappears later in exhaustion (Mann (23), Luxemburg (22)) Goldscheider and Flatau (12, 13), and Sjoeval (32) confirmed this in tetanus Mathes (25) observed the nucleolus of angular shape as I did, the objection, that it might be the deceptive appearance caused by the granules above or beneath the nucleolus, could not come in question in my cases

As a most peculiar manifestation of the nucleus figure 3 was presented, the nucleus is very large compared with the size of the cell body, the nuclear membrane is marked sharply and the nucleolus itself is small and shows an ellipsoid shape Besides that there is a deeply blue-stained substance of triangular shape and the remaining space of the nucleus is filled with compact acidophile substance The nucleus shown in figure 6 might come under



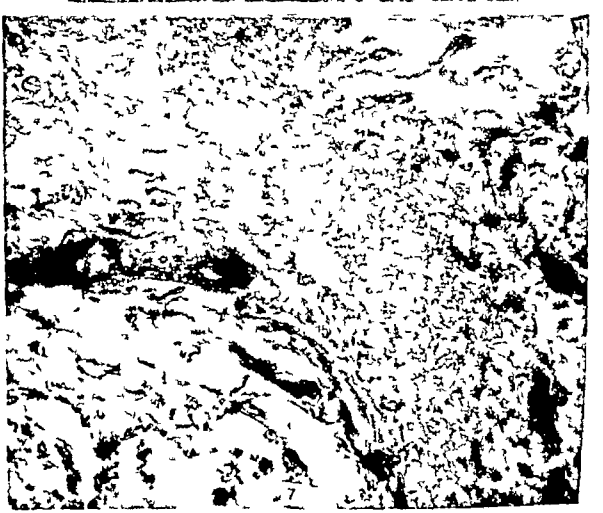


Fig. 6 and 7 Levaditi preparation (fatigued)

the same category of nuclear change, we observe here besides the nucleolus a rod-shaped substance which shows a staining reaction similar to that of the nucleolus, although I am not sure about this, as it is from a Levaditi preparation As far as I know, no such picture of the nucleus has been described before, figure 8 of Dolley's (7) publication demonstrates a cell, the nucleus of which shows a deeply stained spheroidal substance besides the nucleolus, but Dolley did not mention anything about that in the text or in the description of the plate Whether this kind of manifestation of the nucleus is to be regarded as the alteration of double nucleolus, which latter is met not infrequently, or can be attributed to a special appearance of the nucleus in fatigue, I cannot tell

Holmgren (17), Sjoeval (32), and others found the stainable substance massed about the nuclear membrane, and forming either an irregular or a complete ring I also observed the same phenomenon in many cases (fig 3), the nuclear membrane was outlined by a delicate blue-stained line or a large mass of stainable substance in a different portion of its circumference, giving the picture of a half-moon Sjoeval and Holmgren interpreted this as a restitution phenomenon of the tigroid substance, Dolley (7) also regarded it as a sign of greater nuclear activity I agree with the opinion of these authors Holmgren (17) observed besides, that the nucleolus and the nuclear granulation emigrate from the nucleus into the cell body The emigration of the nucleolus never came to my observation, the case, however, from which figure 4 was reproduced may indicate the emigration of stainable substance from the nucleus into the cell body The nucleus as well as the cell body is swollen in this case, and the nucleolus is also extremely swollen, and we find many blue-stained granules going out of the nucleus into the cell protoplasm On the other hand, I found also the accumulation of the acidophile substance in the nucleus (fig 5) On the basis of these findings I should agree also with Holmgren, who came to a conclusion that a mutual interchange of substance takes place between nucleus and cell protoplasm in activity On the ground of Richard Hertwig's doctrine of nucleus-plasm ratio, Dolley (6, 7, 8, 9) measured the size of cell

body and nucleus of nerve cells in activity and divided the cells into many stages of alteration As I did not undertake the measurement of the cell body and nucleus, I will not go further into details of Dolley's work, but his method of division is, as he himself admitted, an arbitrary one, and I found many cells which cannot be assigned to any of his stages Furthermore, I am afraid that in his interpretation of things, facts are linked with hypothetical considerations which are not directly observable I will be satisfied in the present work, if I can make sure that the Mauthner cell, the synapse of which is the material of this study, manifests appreciable changes in fatigue

THE HISTOLOGICAL MANIFESTATIONS OF THE SYNAPSE IN ACTIVITY

In these experiments which consist in forced activity, although it goes far beyond the physiological limit, attention was directed from the first, not only to the nervous constituents of the synapse directly, but also and especially to the manifestations in the glia tissue, which shows the changes of the functioning nerve tissue in an indirect way It was hoped that through the study of the histological manifestations in fatigue some light would be thrown upon the problem, concerning the function of the neuroglia cells in the state of physiological activity I shall first describe the findings in the synapse of the Mauthner cell in fatigue and later go over to the consideration of the significance of the manifestations

A Manifestations in the Pericellular Reticular Structure of the Synapse

The glia reticulum of the axone cap and of the cell surface presented itself in fatigue in a more or less advanced stage of deviation from its normal configuration In the Levaditi preparations which bring out the net figure very clearly and sharply in a dark brown or black color, the alteration of the net configuration is most distinctly noticeable Figures 3 and 4 of my recent publication (24) demonstrate the normal condition of the glia reticulum in the Levaditi preparation The Golgi network, which is visible on the cell surface (3) as well as in the axone cap (4),

stands out sharply, and the net beams are demonstrated in rigid dark lines

In many cases of fatigue this net configuration appears more or less irregular and less sharply marked The net beams present themselves swollen and thick here and there, in other places they are thinner than usual and in some places even broken up The substance of the net beams looks loose and less compact and in the extreme state of decay the net beams are reduced to variably large amorphous corpuscles and look not unlike silver precipitates distributed irregularly in the synapse

Figure 7 was reproduced from the Levaditi preparation of a fatigue case, it shows the surface section of a slightly shrunken Mauthner cell and the cell surface is covered partially with the Golgi network The reticular structure of the synapse stands in this case in a more advanced state of alteration The spheroidal or star-shaped structures, which correspond to the nervous terminal feet, are demonstrated very distinctly in the nodal points of the Golgi network (especially clear in the lower part of the figure) Now, the net beams connecting these terminal feet with each other are in part marked tolerably sharply, but most of them are swollen and are not clean cut Some others look, on the contrary, very thin and loose or even broken up Here and there we find the terminal feet, which lie isolated on the cell surface, as a consequence of the breaking up of the radiating net beams The reticulum of the axone cap (upper part of the figure) is in a similar state of alteration, the net beams are extremely swollen and loosened and appear thick The reticular figure is partly well preserved, although we find here and there the decay of the net beams

Figure 8 was produced from another case prepared by means of Levaditi's method, the Golgi network in the axone cap as well as on the cell surface shows essentially similar changes The net figure here and there is preserved tolerably well, but the meshes are irregular as compared with those in the resting condition In other parts of the synapse the net beams show a more or less advanced state of alteration and some beams are really reduced to amorphous black fragments

The above described findings were repeated in a different intensity in many cases of fatigue by means of Levaditi's method, although there were several cases with negative findings in the synapse It must be emphasized here that in the resting control animal the pericellular reticular structure was always brought out clearly and sharply Of the manifestations of the glia cells in the synapse I shall give a more precise description later As espe-

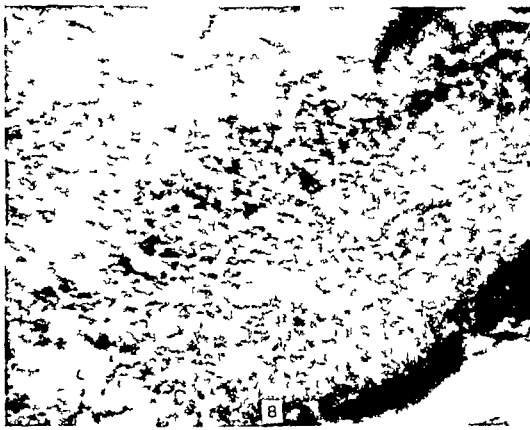


Fig 8 Levaditi preparation (fatigued)

cially worthy of note here, I want to discuss the relation between the glia cells and the reticular glia structure The protoplasm and the protoplasmatic processes of the neuroglia cells increase and swell in mass, as will be mentioned below Some processes show their relation to the reticulum even in their swollen condition, but some of the processes are no longer connected with the glia reticulum Some of them fall to pieces so that we find protoplasm masses of different shape and size around the glia cells

Some glia cells even lie freely in and about the synapse, to this I shall come back again

In the thionin-eosin preparations of the formol-Zenker material it is very difficult to find such delicate alterations of the reticular structure In a slight alteration it is almost impossible to distinguish the difference between the resting condition and the fatigue case, so far as the net figure is concerned In the most advanced stage of alteration, however we are able to find similar manifestations as those described in Levaditi preparations, although it is not so easy to see as in these preparations Figures 2 and 5 were reproduced from thionin-eosin preparations In figure 2, which demonstrates the resting condition, we find regularly arranged dark points around the cell, which evidently represent the net beams of the pericellular reticulum In the fatigued cell (fig 5) we find around the cell body a number of similar dark points, which are, however, scattered without any order at the cell periphery The microscopic observation revealed clearly a change of pericellular network similar to that described in Levaditi preparations, the net beams were in part swollen and others broken up The findings of the glia cells I shall describe later In the Heidenhain preparations and other preparations a similar manifestation of the reticular structure was observable, although it is not so clearly demonstrable as in the Levaditi preparations so that we can find the alteration only in a far advanced state

B Manifestations of the neuroglia cells in the synapse

Among the neuroglia cells of the synapse of the Mauthner cell I found in many cases Alzheimer's amoeboid glia cells (figs 9, 10, 11, 12, and 14) These amoeboid glia cells, as characterized by Alzheimer (1), show a large protoplasmatic cell body with a special morphological structure and small dark nuclei, and in typical

Figs 9, 10 and 14 Thionin-eosin preparation (fatigued)
Fig 11 Acid fuchsin-light green preparation (fatigued)
Fig 12 Mallory preparation (fatigued)
Fig 13 Scharlach R stain (fatigued)

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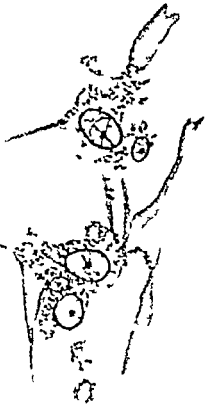

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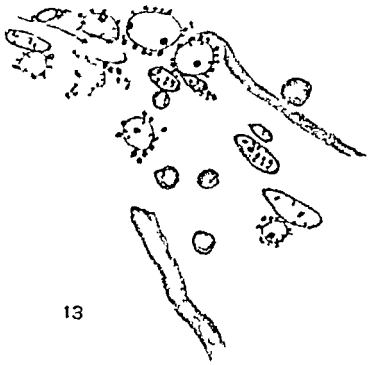

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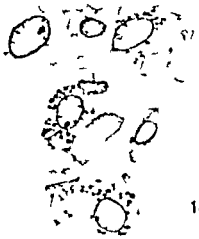

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forms they have striking resemblance to an amoeba Besides the typical forms I found many others which do not resemble amoeba

Before I pass to the description of the amoeboid glia cells, I will pay some attention to the manifestations of glia cells which go hand in hand with the appearance of the former Although not so numerous, we find glia cells in every stage of regressive and progressive change (figs 9, 10, and 14) The regressive nuclei appear sometimes extremely swollen and pale and at other times they show a zigzag shape and deep stain The homogeneous stain of the nucleus and protoplasm, the breaking up of the nucleus into spherules or small masses are other histological properties of the regressive nuclei Furthermore, I observed in the same sections production of young amoeboid glia cell, karyokinesis of the glia nucleus was found now and then, and amitosis of the nucleus, observable occasionally in the physiological condition, seems to appear oftener in fatigue preparations (fig 6)

In young amoeboid glia cells the shape of the cell body is simple and we find sharply marked protoplasm around the nucleus In older cells, however, the protoplasm grows larger and sends processes of irregular shape in different directions The process itself has at first a simple shape, but later it shows a more or less complicated shape, sometimes I observed that the glia cells send the processes to nerve fibers or capillaries, holding the latter between them ramifications Generally speaking, the amoeboid glia cells were found relatively more numerous near the blood-vessels than in the other parts of the synapse In young amoeboid glia cells the protoplasm is first quite homogeneous, but in the further course of life many kinds of manifestation become noticeable in the cell body

In the Mallory preparations (fig 12) the protoplasm of large amoeboid glia cells contains variously large vacuoles, the size of the vacuoles varies considerably, but generally speaking they are not very large in my preparations The number of the vacuoles is also variable with the size and age of the cell The content of the vacuoles is quite clear in my preparations, I assume that these vacuoles are lipoid cysts, the content of which was extracted in the process of embedding In my thionin-eosin preparations

(fig 10) and fuchsin light green preparations I could also find those vacuoles Besides these I observed in the Mallory preparations dark violet or blue-stained granules in the cell protoplasm There is no doubt that these granules are identical with the methyl blue granules of Alzheimer (1) This kind of granules varies considerably in size but in any one cell they are in general of similar size as Alzheimer described With the production of this kind of granule the loosening of the protoplasm structure of the amoeboid glia cells takes place In the acid-fuchsin-light green preparations in which the methyl-blue granules cannot be brought out the cell body shows a granular or bubble-like appearance in this stage At the same time marked changes become noticeable in some nuclei they are stained either homogeneously deeply or remarkably pale Even the neuroglia cells in the synapse which are not in possession of the proper attributes of an amoeboid glia cell but have long narrow processes instead of a large protoplasm mass sometimes display alterations, then the processes look as though they were dissolved into granules, which show the same staining reaction as the methyl blue granules

In the acid-fuchsin-light green preparations (fig 11) the amoeboid glia cells were brought out very clearly in the evenly green-stained cell protoplasm the Alzheimer fuchsinophile granules appeared as large red spherules The number of these granules varied in different cells some large and old cells have granules scattered through the whole protoplasm The sizes of the granules are almost equal and the considerable size distinguishes these granules from the fine fuchsinophile granules, which are only occasionally observable in the normal glia cells As already remarked I found also in this preparation vacuoles of different size in the cell body of the amoeboid glia cells The contents of these vacuoles in my preparations were always clear the large lipoid cysts with yellow substance described by Alzheimer (1) did not come to my observation The Alzheimer light green granules were not observed either in my preparations

In my thionin-eosin preparations (fig 10) of formol-Zenker material I found also a number of typical as well as atypical amoeboid

glia cells, which were met more frequently around the blood-vessels than in the other parts of the synapse Large and old cells showed vacuoles of different size in their bodies Some of these glia cells showed another kind of granules which were demonstrated by means of the thionin-eosin stain in a characteristic metachromatic or more or less blue-violet color The granules fill the cell body as well as the processes the size is variable, but in any one cell they are of almost equal size The shape of these granules is round or that of irregular lumps Another characteristic of this kind of granules is that in the illumination by electric light they are especially beautifully observable In the space around the blood-vessels and also in other parts of the synapse I often noticed a group of these granules, this is to be interpreted as the section of a cell or its process, bearing this kind of granules As far as my observation went, these granules do not lie freely in the tissue

What is the nature of these granules? Reich (27, 28, 29, 30) demonstrated in the Schwann cells of the peripheral nerve fibers rod- or comma-shaped fairly large granules (π-granules), which were brought out in a characteristic metachromatic stain by means of thionin, toluidin-blue or kresyl-violet, and he identified these granules with the protagon of Liebreich on account of the similarity of the staining reaction and of the solubility in warm alcohol (45°) and in warm ether He found, moreover, that they are soluble also in warm xylol, that they are not at all stainable in acid stains, and also that they are especially beautifully observable in the illumination by electric light

Later, in certain pathological conditions, Alzheimer (1) demonstrated in the neurogolia cells granules which gave a characteristic metachromatic basophile stain by means of toluidin-blue and thionin, he identified these granules with the π-granules of Reich and called them metachromatic basophile granules, although the granules differed somewhat from the π-granules morphologically I did not test the properties of solubility of the granules, which I observed in the glia cells, but on the basis of their staining reaction and morphological characteristics I assume that they are identical with the metachromatic basophile

granules of Alzheimer Whether these granules consist of protagon or not, is another question, recent studies raised doubt against the real existence of protagon as a uniform substance (Rosenheim Tebb, Thudicum, cited in (18)) I should add here that I always used bergamot oil instead of xylol in the process of paraffin embedding

Alzheimer (1) declared that he did not find, or he found at the most only indications of, these granules in the amoeboid glia cells but as far as my observation went I found a number of amoeboid glia cells with this kind of granules The finding that these granules are observable in the cells of blood-vessels, as will be described later, and in the glia cells around the blood vessels relatively more numerously, and the fact that in Scharlach stain fat drops are found in those cells as will be related below, make one assume that they are transported toward the blood vessels and that these granules give rise to the production of fat as Alzheimer did Reich assumed that the appearance of this kind of granules has a relation to the decay of the myelin sheath, according to Alzheimer (1), it is not, however, necessary for the appearance of this kind of granules As the neuroglia cells of the synapse of Mauthner's cell lie mostly at the border of the axone cap where the nerve fibers lose their myelin sheaths I cannot decide this question from my own observation Moreover as the granules appear only in fatigue, it is probable that they have something to do with a pathological nutrition condition of the nerve tissue the fact that they are a catabolism product is acceptable because they are transported toward the blood-vessel and it is also probable that they are changed into fat just like the other catabolism products

On the basis of the above-described facts it is quite clear that in fatigue a number of amoeboid glia cells are produced in and about the synapse which carry different kinds of catabolism products in their cell body as well as in their processes As already repeated I found these amoeboid glia cells relatively more numerous around the capillaries in and about the synapse It was also remarked that many a conglomeration of metachromatic basophile granules was demonstrated around the blood vessels

with a different size and shape (fig 10) This was also the case with the methyl-blue granules and the fuchsinophile granules All these granules were embedded in the evenly and lightly stained ground substance, which is to be interpreted as the section of the cell body or the process of the glia cell As far as my observation reached, these granules did not lie free in the space around the blood-vessel or in the tissue

The cells of the adventitia of blood-vessels in the normal brain show very little protoplasm around their nuclei, but in fatigue cases, when a number of amoeboid glia cells are present around the vessels, they show a larger mass of protoplasm In the thionin-eosin preparation I observed occasionally the metachromatic basophileg ranules in them Scharlach R (fig 13) revealed many fat drops in the latter It must be added here that in the neuroglia cells around the vessel and in the synapse fat drops were demonstrated within the protoplasm According to my observation, there was, however, very little fat lying in the tissue or in the space around the vessel

THE 'FUELLKOERPERCHEN' OF ALZHEIMER

After the description of the changes of the glious reticulum and the glia cells of the synapse, one more manifestation, which goes hand in hand with the appearance of the amoeboid glia cells is to be mentioned I have already described that in sections in which a number of typical amoeboid glia cells were observable, many a protoplasm mass of different size and shape appears in the perivascular space and in the other parts of the synapse These protoplasm masses were found to be sometimes homogeneous and sometimes granular and they occurred usually near the amoeboid glia cells Some of these masses must be regarded as the section of the cell body or the process of an amoeboid glia cell (fig 10), but some of them show evidently that they were cast off from the body of the amoeboid glia cells (fig 15) It was also related above that the processes of the glia cells, which are not in possession of the proper attributes of an amoeboid glia cell are broken up in their substance and are reduced to fragments I assume that I

can homologize these protoplasm pieces with the 'Fuellkoerperchen' of Alzheimer (1), as far as the origin of these corpuscles is concerned, I agree with Alzheimer, in so far as the reticular beams of glia tissue swell and are loosened in their substance so that finally they fall here and there to pieces The probability of a post-mortem alteration cannot come into consideration, it must be emphasized here that the fish brains were always fixed in their fresh condition

THE RELATION OF THE AMOEBOID GLIA CELLS TO THE GLIA RETICULUM

As stated before, the glia reticulum of the synapse of the Mauthner cell in fatigue was found in a more or less advanced state of deviation from its physiological configuration The net figure appeared less sharp and the meshes were found more irregular, the net beams were observed either swollen or lessened in thickness The substance of the net beams appeared loosened and in the state of extreme deterioration the net beams were here and there broken up and reduced to fragments so that in some parts of the synapse the net figure was no longer in evidence The question now arises whether these manifestations of the peri cellular reticulum are to be attributed to artefacts caused by the process of preparation of the sections or to be claimed as ante-mortem phenomena caused by the over activity The following circumstances speak explicitly for the latter, first, I got the same results in different kinds of preparations, second, I did not find any case of resting control fish, in which a similar picture of the reticulum was observed, and, third I noticed a number of amoeboid glia cells with various catabolism products and the so-called 'Fuellkoerperchen It must be emphasized here that the nuclei of the glia cells of the synapse showed not only regressive but also progressive changes

Buscaino (5), Rosental (31) and others studied the postmortem appearance of the amoeboid glia cells Wohlwill (35) declared that through the postmortem decay of neuroglia tissue glia cells take occasionally the amoeboid appearance and the occurrence of the methyl blue granules and the Fuellkoerperchen does not

always indicate the previous existence of amoeboid glia cells In the present work all the fish brains, both normal and fatigue, were placed in the fixing solutions in their fresh condition, and the likelihood of postmortem production of the amoeboid glia cells cannot at all come under consideration Attention must especially be called to the fact that in all my control preparations not a single amoeboid glia cell did come to my observation in and about the synapse

What would then be the mechanism of the breaking up of the glia reticulum? It is very hard to answer this question definitely Eisath (10), who studied and demonstrated the protoplasmatic glia structure very well by his own method, suggested that in the sections in which amoeboid glia cells occurred either in the marrow only or in both the marrow and the cortex, the glia cells with delicately arborized protoplasm processes do not come to observation Alzheimer (1) also made the same observation, instead of the glia cells with arborized protoplasm processes, he found by means of the same method glia cells with a little increased plasm without any process or those with large cell body carrying different kinds of granules He took for granted that with the appearance of the amoeboid glia cells the other glia structure also sustain some alteration On another occasion Alzheimer (1) made the observation that in a cortex, which showed no glia cells with protoplasmatic processes by means of Mallory's method, the Golgi method revealed such cells with processes On the ground of this finding, he carefully expressed his opinion, declaring that the processes did not here go into decay or were not withdrawn by the cells, but merely did not show the affinity to Mallory's hematoxylin

Now, as already remarked, the processes of the glia cells of the synapse arborize and unite into a uniform glia reticulum in the synapse This condition can be beautifully demonstrated in the Levaditi preparations In fatigue a number of the glia cells are converted into the amoeboid glia cells and they lie free from the reticular structure of neuroglia tissue, and in this case attention must be called to the fact that some other glia cells in the synapse still show their relation to the reticulum and that I could observe

almost every stage of the dissolution of an amoeboid glia cell from the diffuse glia reticulum Near the amoeboid glia cells the 'Fuellkoerperchen' or the fragments of the Golgi net beams were also observable, as already stated So I came to the conclusion that in the extreme stage of fatigue a number of amoeboid glia cells are produced and are set free from the reticulum Jakob (18) recently described in his article on secondary degeneration the detachment of the glia cells from the diffuse glia reticulum Of course this process took place only slightly and on a small scale in my material, but the mechanism would here be the same At the same time the substance of the reticular structure becomes looser, and finally some of the beams undergo dissolution and fall to pieces so that the above-described alteration of the reticulum takes place What effect the fixing solutions have here on the more or less loosened reticular beams, I can not say but I believe that the mechanism of the breaking up of the glia reticulum could well be interpreted by the above-described facts

As far as I know, the studies of the pathological alteration of the pericellular reticulum are very few, the studies of Eisath (10) and Alzheimer (1) were not especially directed toward the pericellular reticulum, but merely to the glia reticulum in general Besta (4) investigated the behavior of the pericellular reticulum in certain pathological conditions, but his description does not explain the mechanism of the deterioration clearly enough and he did not mention the appearance of the glia cells at all As far as the manifestation of the structure in question in fatigue is concerned, this investigation is unique

THE BIOLOGICAL SIGNIFICANCE OF THE AMOEBOID GLIA CELLS AND THE CONCLUSION FROM THE RESULTS

As far as my observation went and so far as the histological technique used brought out the facts, over activity caused no definite change in the nervous structure of the synapse of the Mauthner cell Considering the nature of the experiment, one should not be surprised at this, moreover, the structures in question are so fine and the results of the technique for those structures are

sometimes so inconstant that one should be very careful to attribute any pathological significance to any slight histological manifestation in those structures The appearance of the amoeboid glia cells may indicate, however, that some catabolism process takes place in and about the Mauthner cell Alzheimer (1), who investigated thoroughly the amoeboid glia cells and the catabolism processes in the nerve tissue, said about the cases in which a number of amoeboid glia cells were found without any finding on the side of nervous structure, that some catabolism products which escape the microscopical demonstration at the present time would be produced on account of the disturbance of the nutrition of the nerve tissue The finding of the amoeboid glia cells in the synapse might show that in over-activity some catabolism products are produced as the effect of pathological nutrition condition or owing to dilapidation of the nervous structure, not demonstrable at the present time These would stimulate the formation of the amoeboid glia cells to serve as scavengers That postmortem production of the amoeboid glia cells cannot come under consideration, I already remarked Rosenthal (31), who wanted to interpret the appearance of amoeboid glia cells with methyl-blue granules as a sign of necrobiosis of neuroglia tissue, regarded the formation of the amoeboid glia cells with fuchsinophile granules as that of increased scavenging activity, the amoeboid cells with these granules were also found in fatigue, as remarked According to Wohlwill (34), different kinds of diseases which show the amoeboid glia cells have edema as their common cause The question whether over-activity causes edema in the region of the synapse and a swelling process of the glia cells can come under consideration or not, must be left undecided here So far I may assume that in over-activity a catabolism process in a wide sense takes place in the synapse, which comes under the fourth category of catabolism processes of Alzheimer (2) But I cannot state definitely whether the catabolism products come only from the synapse or from both the synapse and the cell, the latter appears to me more probable

The question whether the amoeboid glia cells come from newly produced glia cells or from those which were present in the synapse, is very hard to answer, but the finding of many amoeboid glia cells in spite of very little increase of the glia cells may indicate that at least some of the old glia cells give rise to amoeboid glia cells As far as the transition of one kind of catabolism product into another within the protoplasm of the amoeboid glia cells is concerned, I cannot add much to the description of Alzheimer (1) The fact that we find the amoeboid glia cells with different granules and lipoid substance relatively more numerous around the blood-vessels than the other parts indicates that the catabolism products are assimilated into different granules by the amoeboid glia cells and carried to the blood-vessels and deposited in the cells of blood-vessels as fat and later are gradually disposed of

In my experiments I could not find a single case in which the picture of the 'neuronophagia' of the Mauthner cell was observed, this might be interpreted to mean that the alteration of the cell body did not go so far in over-activity Dolley (8) described cell death as the effect of over-activity, it is rather strange to me that no attention was directed to the changes of the glia cells in his article

SUMMARY

Careful investigation of the cell body as well as the synapse of the fatigued Mauthner cell in Ameiurus revealed a number of interesting findings, which can be summarized as follows

1 The cell body was found either swollen or shrunken, the turgescence was regarded as the result of over activity and the shrinkage as that of exhaustion

2 The Nissl substance was in a more or less advanced stage of chromatolysis and thereby the cytoplasm was stained variously deeply On the border of the nucleus a mass of stainable substance was observed as the restitution phenomenon of the Nissl bodies on the side of the nucleus It was also accepted that a mutual interchange of substance occurs between nucleus and protoplasm in activity

3 The nucleus was also either swollen or shrunken in the swollen nerve cell it was sometimes removed to one side of the cell body The nucleolus was also found swollen sometimes and other times shrunken and it was of angular or otherwise irregular shape

4 In the nervous structure of the synapse no definite alteration could be brought out but the synapse showed a number of amoeboid glia cells with methyl-blue fuchsinophil and metachromatic basophile granules Also fat drops were demonstrated in the glia cells and in the cells of the blood-vessels

5 The reticular glia structure of the synapse appeared in many cases of fatigue in more or less advanced deviation from its normal configuration and even broken up in some parts, this was interpreted as the result of the detachment of the amoeboid glia cells from the reticulum as also the effect of the loosening and dissolution of the net beams

6 The appearance of the amoeboid glia cells showed that some catabolism process occurs in the synapse as the effect of pathological nutrition conditions in fatigue

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Resumido por C. Judson Herrick, por el autor, O. Van der Stricht.

El desarrollo de las células de los pilares, el espacio del tunel y los espacios de Nuel del órgano de Corti

El espacio del tunel se desarrolla alrededor del fascículo del nervio espiral, el cual camina entre las porciones nucleadas de las células internas y externas de los pilares. En su origen es una hendidura intercelular cuyo contenido líquido es elaborado por el citoplasma vacuolar de las células de los pilares y se vierte en el espacio adyacente. Algunas partes de este protoplasma secretor sufren un proceso de citolisis, de tal modo que la hendidura crece y su contenido líquido aumenta en cantidad a sus expensas. El autor describe con detalle el desarrollo ulterior de las células de los pilares y sus cabezas. El primer espacio de Nuel aparece en forma de una hendidura longitudinal situada entre los pilares externos y las células ciliadas externas y en su interior se acumula el líquido segregado por los pilares externos. En las superficies laterales de estos últimos se proyectan vesículas de secreción claras, las cuales experimentan un proceso de citolisis y liquefacción. Las células externas de los pilares verifican una emigración embrionaria desde la primera fila de células ciliadas externas hacia las células internas de dichos pilares. El autor describe el desarrollo del segundo, tercero y cuarto espacio de Nuel. El contenido líquido del tunel y el del primer espacio de Nuel se mezclan a través de hendiduras existentes entre los pilares externos y comunican con las de los segundo, tercero y cuarto espacio de Nuel. El líquido de todos los espacios de Nuel está separado de la endolinfa del conducto coclear por los techos, muy delgados, de estos intersticios. Tales estructuras realizan, indudablemente, la propagación de las ondas vibratorias desde la membrana basilar hasta la membrana tectoria, contenida en el canal coclear.

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THE DEVELOPMENT OF THE PILLAR CELLS TUNNEL SPACE, AND NUEL'S SPACES IN THE ORGAN OF CORTI

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EIGHTEEN FIGURES

CONTENTS

INTRODUCTION

In spite of numerous thorough and exhaustive investigations concerning the earliest stages of development of the organ of Corti our knowledge of the origin of the tunnel space is still very limited and vague. This is true also of the formation of the heads and cephalic appendages of the pillar cells and almost nothing is known concerning the origin of the so-called spaces of Nuel. This observer (78) described in the organ of Corti in adult mammals a system of intercellular channels and his findings have been confirmed by Retzius (84) and other more recent authors. These spaces or channels are situated between

the outer rods of Corti and the neighboring outer hair cells and between the three rows of outer acoustic elements. They contain fluid and are traversed by the phalanx processes of the cells of Deiters, intercommunicating through clefts between the sensory elements and communicating with the tunnel space through interstices between the outer pillars. The view taken by Nuel, that they communicate with the lumen of the cochlea "par l'intermédiaire de lacunes en forme de rames de la membrane réticulaire" must be regarded as erroneous.

The development of the tunnel space between the two spiral rows of rods of Corti appears to be very difficult of observation. Indeed most authors referring to its appearance in embryonic material state that it originates in the form of a narrow cleft between the inner and outer pillars but give no details concerning the significance of this primitive interstice. Is it intercellular or intracellular? From what source is derived the fluid contained within the cleft? Those observers who clearly specify that the space appears between two neighboring pillars, as do Gottstein (72), Retzius (84), Vermeuwe ('05), N. Van der Stricht (08) and Hardesty (15), give no explanation as to the origin of its fluid. Alluding to the development of the space in rabbits two days after birth, Retzius states (p. 303):

Von besonderer Bedeutung ist nun die enge Spalte, welche zwischen den beiden Pfeilerzellen nahezu ungefähr in der Mitte der Zellenhöhe, nach oben vom spiralen Nervenbündel entstanden ist und den Anfang des Tunnelraums darstellt, die Spalte ist in der Basalwindung—wo auch noch eine geringe Neigung der Pfeilerzellen nach aussen hin vorhanden ist—noch viel weiter entwickelt, und man sieht hier deutlich, dass die durch Einziehung (Verdünnung) der beiderseitigen Pfeilerzellen entstanden ist. Gleichzeitig ist aber auch die Anlage der Pfeiler in den Zellen als hellglänzende Streifen nunmehr wahrnehmbar. Nach aussen von den äussersten Pfeilerzellen sieht man deutlich auch die Anlage der Nuelschen Räume.

According to Vermeuwe, the tunnel space is produced by the separation of the bases of the two pillar cells, due to elongation of the pillars, increase in size of the nuclei and chiefly by the extension of the subjacent basilar membrane. Referring to the trend of the spiral organ of Corti towards the axis of the cochlea, Har-

desty ('15, p 52) states "The normal spaces between the elements of the spiral organ, including the large Nuel's space, no doubt result in part from this movement of the organ outward" Other authors, Rosenburg ('68), Boettcher ('69), and Pritchard ('76) describe two neighboring inner and outer pillars as derived from a single original cell the nucleus of which divides in two, and by a process of liquefaction of the undivided cytoplasm, the tunnel space is produced within it This space is originally intracellular and its fluid is a protoplasmic product Rickenbacher ('01, p 402) seemingly ascribes a similar origin to the fluid of the space of Nuel in the adult guinea-pig "Bei der Schnecke des ausgewachsenen Tieres hat der Prozess der Verflüssigung zur Bildung des Nuelschen Intercellularraume und des Leiterepithels gefuhrt" According to Kishi ('02), the tunnel space is due to the spiral course of the nerve fibers after they have passed through interstices between the inner pillar cells The formation of tunnel and intercellular clefts is considered by Held ('09) to be the result of 'ungleichen Wachstumbewegungen' of different epithelial cells His so-called 'outer tunnel,' the spaces between the outer hair cells, and the space of Nuel outside the outer pillars are sheer intercellular channels, 'reine Intercellularspalten,' but the tunnel between the pillars is originally intracellular

Eine reine intrazellular Spalt, da die ersten Nervenfasern die hier spiralig abbiegen und weiter ziehen nicht in der Zwischengrenze zwischen Aussen und Innenpfeiler liegen sondern im Protoplasma der Innenpfeilerzellen randständig eingebettet sind was auch für die unten den inneren sowie äusseren Haarzellen resp zwischen den Deiterschenzellen und in ihren Interzellularbrücken gelegenen Formation eines intraepithelialen Nervusplexus gilt

The development of the tunnel and the pillar cells is closely connected with the formation of the pillar heads the appearance of the 'head plates' of the inner pillars the phalanx processes of the outer pillars and the extension of the membrana reticularis The superficial structures of the rods of Corti in adult mammals have been exhaustively investigated by many observers Max Schultze ('58) Koelliker ('59) Boettcher ('59–72) Deiters ('60),

Hensen ('63–'71) Gottstein ('70–'72), Nuel ('78) Tafani ('84) Retzius ('84) and by most of the more recent authors but the appearance and extension of these structures and the mechanical factors taking part in their formation require more careful study. N. Van der Stricht has shown that the head-plate of the inner pillar is originally represented by a very small square field the apex of the cell which becomes fibrillated and extends over the enlarging head of the outer pillar, the former undergoing great pressure from the latter. The outer pillar cells originally belong to the first spiral row of outer sensory elements. As development advances they are pressed out from this row towards the inner rods of Corti and form a new row of outer rods, the pieces of which always remain fixed between those of the outer acoustic elements of the first row. Hence there persists an apical segment of the outer pillar, which runs obliquely from the apex or phalanx of the cell, downward and inward toward the future head of the pillar. This oblique process contains a bundle of fibrils which, issuing from the head, passes between two outer acoustic elements and spreads out upon the phalanx—the head-plate of the outer pillar. By enlargement of the head, the fibrillar bundle gradually acquires a more horizontal position. Held ('09, p. 109) seemingly ascribes the head-plate of the inner pillar not only to the apex, but also to the superficial portion of the cell "der obere Zellteil welche die Faserrohre enthalt," and which is pressed flat from the developing head of the outer pillar. Although he did not recognize the original position of the outer pillar cells within the first row of outer acoustic elements he nevertheless observes the squeezing of their 'Kopfplatte' which becomes thinner from compression between two hair cells, and also of the bundle of fibrils, which at first run obliquely then at right angles to the intermediate piece of the outer pillar due to pressure from the elongating pillar cell.

In the present paper the appearance of the tunnel space, the development of the heads and cephalic appendages of the pillar cells and the formation of the Nuel spaces will be dealt with in order

DESCRIPTIVE

Appearance of the tunnel space

Sections tangential to the surface of the organ of Corti, and always somewhat oblique, affect transversely series of neighboring inner and outer pillars at various and successive levels of their length, from the superficial membrana reticularis toward the basilar membrane (fig 1) As illustrated in figures 1 and 2, one may distinguish in the prismatic lamellar pillars three portions, although they are not sharply marked off a basal or nucleated part, the largest, which is lamellar in shape or flattened out in a radial direction from mutual compression, an intermediate part, and a superficial part, the narrowest, which is compressed between the inner and outer hair cells, and hence more or less flattened out in a spiral direction (*ip* and *op*) The basal and intermediate portions are each made up of two cytoplasmic zones, the larger being clear and vacuolated and occupying the area of the cell body close to the future tunnel, the smaller compact and fibrillated, and occupying the axial side of the inner pillar and the lateral side of the outer pillar The superficial segment of the two rods of Corti contains no vacuolated protoplasm, it consists of a more homogenous compact cytoplasm, which in the inner pillar is traversed by a bundle (fig 2, *ip*) or a tubule (fig 3, *ip*) of fibrils, and in the outer, encloses a bundle of fibrils which pass between neighboring outer hair cells and give rise to a small band, the phalanx process of the outer pillar (figs 1, 2 and 3, *oph*) connected with the superficial apex of the cell, the phalanx In the adult organ a part of this fibrillar bundle is a characteristic constituent of the superficial portion of the head and thus its early presence in a definite portion of the outer pillar is very important in enabling one to determine, from the earliest stages of development, a very narrow but long superficial portion of the cell (figs 1, 2, and 3 *op*), which later enlarges and becomes transformed into a part of the bulky head It is also obvious that the adjoining portion of the inner pillar which in figures 1 2 and 3 is in close contact with this future head of the outer pillar must be con-

sidered is the segment which will become converted into the so-called head of the inner pillar

The outlines of all the pillars are very sharp, not only between the cells of the same row but also between the neighboring elements of the two rows While at the level of the superficial segments this outline is represented by an intercellular material (figs 1 and 3 *th*) which in its staining capacity and chemical constitution agrees with that of the superficial terminal bars, between the two lower segments of the pillars it is composed of a paler more fluid or true intercellular cement, in addition to which a very thin superficial cytoplasmic film can be brought into view This outline and film are lacking along the axial surfaces of the inner pillars and the lateral surfaces of the outer The spiral nerve bundle (V^{n}) occupies an intercellular position between the nucleated parts of the outer and inner pillars, sometimes encroaching somewhat upon the lower interstice which separates their intermediate portions The nuclei of the pillar cells are surrounded by vacuolated cytoplasm The nuclei of the inner pillars are much smaller than those of the outer and much more flattened out radially

When the tunnel space is about to appear, there occurs a characteristic alteration in the cytoplasm adjacent to this future cleft, the vacuoles running together and thus increasing in size (figs 1 and 2, *t*) A common vacuolated mass soon appears (figs 3 and 4, *t*) at certain places it remains fused with the cell body from which it is derived, at others it is independent, so that one cannot determine to which of the neighboring pillars it belongs From this moment there exists a narrow intercellular cleft, filled with a small amount of extracellular, vacuolated material, a common mass which doubtless represents the first trace of the intratunnelar fluid, and which gradually increases in quantity by the coalescence of adjoining portions, partly incorporated in the original pillar and partly free or extracellular Although from the earliest stages of the appearance of the space small extracellular, vacuolated masses can be found between the intermediate segments of the pillars (fig 4, *t*), the larger part of the tunnel is generally seen around and close to the spiral

nerve bundle, that is to say, between the nucleated portions of the inner and outer rods of Corti (fig 5, *t*, *T*), never "ungefahr in der Mitte des Zellenhohe," as Retzius asserts and many investigators illustrate It is a perinervous space Later it extends between the intermediate portions of the pillar cells

According to this description, the tunnel space must be held to be a true intercellular cleft, the fluid contents of which are developed by a process of secretion from the neighboring parts of the pillars and a simultaneous partial cytolysis of the latter The space enlarges at the expense of the cell bodies In the earliest stages of development of the organ of Corti there appears in the pillars not only a fibrillated sustentacular apparatus related to their function of support, but also a large, clear, vacuolated cytoplasm the bulk, of their cell bodies This portion of the protoplasm is glandular in nature, and from the blood plasma of the subjacent vas spirale (fig 1, *vs*), it derives its nutritive material, which is elaborated and converted into clear vacuoles The products of secretion are discharged along with a partial liquefaction of the surrounding cytoplasm

During the extension of the tunnel space the superficial segments of the outer pillars undergo considerable enlargement, and their radial diameters soon correspond to those of the intermediate portions (fig 4, *ohd*) At that time the process of cytolysis obviously extends along these segments (figs 4 and 5 *t*) from below upward, involving a rapid reduction of their radial diameters The intermediate segment of the outer and inner pillars, previously broad and formed of a small fibrillated part and a large vacuolated portion next to the future cleft, becomes gradually converted into a slender band the so-called 'body' of the pillar In figure 7 (*opb*) these bodies are shown cut at successive levels through fifteen outer pillars In their lower portion as seen in nine sections, they are reduced to thin cylindrical fibrillar strands, part of their apparatus of support, from around which the clear cytoplasm has disappeared In their upper part, as seen in the next four sections, the pillar bodies are still composed of the original two zones the vacuolated portion having been somewhat reduced Close to the

future heads are seen two sections (connected with sections of inner pillars), the structures of which have undergone no change The process of cytolysis is completed at the level of the first nine elements, it is progressing in the following four and has not yet begun in the last two On comparing these structures with more advanced stages, and especially with those in the adult cochlea, it is plain that the body of the outer pillars acquires its final form and structure by a process of secretion and cytolysis along with the elongation of the intermediate segment In young cats, bats, common and white rats it becomes a slender fibrillated strand, destitute of clear cytoplasm (figs 10, 13', 14, 15, 17 and 18, *opb*) Between the pillar bodies, as already noted by Nuel, are large clefts through which the fluid of the tunnel space and the neighboring space of Nuel intercommunicate

The intermediate portions of the inner pillars undergo similar, but never such marked changes The greater part of their clear cytoplasm disappears, only a very narrow zone of it persisting, so that in young and adult animals the body of the pillar becomes lamellar in shape (fig 18, *ipb*) and flattened out in a spiral direction It is composed of a fibrillar lamella and a thin layer of clear protoplasm (fig 17, *ipb*) Besides the pores traversed by the nerve fibers, no true intercellular clefts sever the inner pillars

Along with these alterations and elongation of the pillar bodies, the tunnel space enlarges gradually but considerably, and very soon its radial diameter surpasses that of the two original clear zones belonging to two contiguous pillars In other words the fluid accumulated within the cleft exceeds the amount of disintegrated protoplasm Indeed the cytolysis occurs in a merocrine glandular cell which, although undergoing partial liquefaction, is able to elaborate new clear secretion products at the expense of material derived from the vas spirale Hence the tunnel fluid is the result not only of a sheer cytolysis, but also of a true elaboration and subsequent discharge In the earliest stages of development the process of cytolysis seems to be more prevalent, since the contents of the cleft are seen in the form of a coagulated, vacuolated mass, afterwards the larger space is

usually filled with a clear uniform fluid, which seems to arise from a more active, true secretion This secretion may continue even in the adult organ, for, according to all the investigators, the nucleus of each pillar cell is surrounded by a clear cytoplasm which extends over the floor of the tunnel This protoplasm is vacuolated and represents the rest of the original bulky, glandular portion of both sustentacular and secreting cells

Development of the heads and the cephalic appendages of the pillar cells

According to the results published in a previous paper (now in press) and those obtained by N Van der Stricht, at the earliest stage of the development of the organ of Corti the outer pillar cells are located within the first spiral row of outer hair cells, and their superficial segments occupy interstices between two neighboring acoustic elements By the rapid enlargement of the latter, these superficial elements are pressed out of the row and pushed towards the inner pillars, although their apices remain fixed between those of the hair cells From this time the inner and outer rods of Corti constitute a scaffolding, which is *made up of two spiral rows of sustentacular elements and is* triangular in shape on vertical section The rapidly enlarging base of the triangle abuts against the basilar membrane, and the apex is interpolated within the superficial membrana reticularis, separating the apices of the supporting and sensory elements of the inner spiral row from the apices of those of the first outer spiral row In a section tangential to the organ of Corti the summit of the scaffolding is represented by a spiral row of very narrow fields, the apices of the inner rods of Corti, separated from one another and from the neighboring fields of the reticular membrane by deeply staining terminal bars, which extend into the depth between the superficial portions of the inner and outer pillar cells Each of these narrow fields contains a diplosome, and will gradually enlarge by a process of compression from the underlying expanding heads of the outer pillars

Outer pillar In the superficial part of the entirely developed outer pillar, as seen in the adult organ of Corti, three different portions are distinguishable 1) The apex or 'phalanx,' forming a part of the membrana reticularis This consists of a lateral, expanded segment (fig 8, *oph*^{1}), which constitutes a portion of the roof of a subjacent intercellular interstice through which comes the phalanx processes of the cells of Deiters of the first row (paper in press), and a medial, constricted segment (*oph*^{11}) lying just between two apices of the outer hair cells of the first row (*oh*^{1}) 2) A fibrillated band or the phalanx process (fig 13^{1}, *oph*^{1}, *oph*^{11}, *oph*^{111}) which runs nearly horizontal and unites the apex to the head 3) The head proper or the enlarged superficial part of the pillar in contact with the inner pillar (figs 13^{11}, 17 and 18 *ohd*) This is a cubical segment, in sections tangential to the surface it is square (fig 13^{111}, *ohd*) or somewhat lengthened out radially (fig 18, *ohd*) Its upper portion is traversed by the fibers of the phalanx process (fig 17 *ohd*), and its larger, lower part by a fibrillar bundle belonging to the body of the pillar (fig 13^{11}, *ohd*) Thus two different fibrillated fascicles spread out and fade off into the head there is no direct continuity between the fibrils of the two bundles (figs 14 and 15 *ohd*)

In the first stage of development which may last until the tunnel space is about to appear and before there is any marked increase in the size of the future head (figs 1 2 and 3 *op*) the three parts of an adult pillar just referred to are recognizable The apex acquires the appearance illustrated in figure 1, *oph* The phalanx process is short and a nearly vertical deeply staining bundle of fibrils (figs 1 and 3 *oph*) which is traceable between the cell bodies of the outer hair cells (*oh*^{1}) and a little deeper between these sensory elements and the future head The future head is a thin tapering part of the pillar, composed of a more or less homogeneous cytoplasm which encloses in its upper two-thirds the rootlets of the phalanx fibrils, and in its lower one-third the summit of the bunch of fibrils of the pillar body (figs 2 and 3 *op*) Indeed, in figures 1, 2 and 3 two or three fields, cross-sections of the future head contain parts of

the two fibrillated bands This rather deep portion of the pillar, situated at the level of the lower poles of the outer hair cells (*oh*^{1}), doubtless belongs to the developing head From this it is evident that the superficial, thin tapering segment of the outer pillar cells which gives rise to both the phalanx process and the head, attains more than one-third (figs 1 and 3, *op*) or about one-half of the entire length of the cell, or about the length of the outer acoustic element (*oh*^{1}), although no distinct demarcation can be observed between the future head and the pillar body

Two other features lend support to this view the existence of an abundant, vacuolated cytoplasm along the intermediate portion of the cell, the future pillar body, which only slightly encroaches upon the lower part of the future head, and the presence of terminal bars or rather true intercellular, obturating partitions These have been observed and termed bandelettes obturantes' by N Van der Stricht ('08) and 'Kittsubstanz or 'Kittlinie' by Held ('09) This material stains intensely with iron hematoxylin like the superficial terminal bars with which it is in continuity, and corresponds to them in nature and chemical constitution It gives rise not to 'lines' or 'bars,' but to true septa, uniting parts of the cells and obturating the subjacent intercellular spaces These partitions exist not only between contiguous developing and definitive heads of inner and outer pillars, but also between the apical surface (that turned toward the apex of the cochlea) and basal surface (that turned toward the base of the cochlea) of the heads of each spiral row On the other hand they are altogether lacking along the medial surfaces of the heads of the inner pillars and the lateral surfaces of those of the outer (figs 1 and 3, *ib*)

The second stage of development is characterized by a rapid enlargement of the future head of the outer pillar (fig 4 *ohd*), so that it reaches the site of the intermediate portion or even surpasses it, when the process of cytolysis progresses along the tunnel space (fig 7, *op*) At first the head remains smaller next to the surface, but soon this portion expands and becomes somewhat larger than the deeper part (fig 1 *ohd*) and acquires

a cubical or prismatic shape, the larger base of which touches the surface of the organ of Corti, its tapering apex blending with the much smaller pillar body In cross-sections the prism is square or quadrangular in shape

During this process of enlargement of the head, many remarkable changes occur 1) A considerable shortening of the head segment (fig 7) as if the compact substance of the lower parts had been pushed upward Moreover, there can be no doubt that at the same time, the vacuolated cytoplasmic zone of the intermediate portion of the pillar extends upward along the primitive head, so that the pillar body becomes longer at the expense of the latter 2) A peculiar transformation of the protoplasm of the heads of the outer and inner pillars, close to and through the agency of the obturator septa Primitively compact, homogeneous or granular, entirely different from the vacuolated or fibrillated cytoplasm above referred to, the protoplasm of the head becomes converted into a denser material, staining intensely with iron hematoxylin These changes occur in succession, first within the heads of the outer (figs 1 and 7, *ohd*) then within those of the inner pillars (fig 8 *ihd*), in proximity of the obturator septa separating their apical from their basal surfaces, later, along the medial surfaces of the heads of the outer pillars, and finally along the lateral surfaces of the heads of the inner pillars, close to the obturator partitions which separate these two elements (fig 9, *ohd* and *ihd*) In sections tangential to the surface of the organ of Corti these altered cytoplasmic portions are seen in the form of deeply staining uniform planoconvex masses, the planar surface of the clump of one head adjoining that of another mass belonging to a contiguous head (fig 11) In reality, each planoconvex clump is the section of a vertical band or semicolumn Thus in each head there appear three semicolumns, which at first are separated from one another but in more advanced stages coalesce to form a single band or imperfect collar open toward the side of the head where the obturator material is lacking (figs 7, 8, and 9) What mechanical factors cause these structures to appear is uncertain It can only be stated that this dense and horny-

like exoplasmic head collar develops and extends in close contact with the intercellular septa, as if the material elaborated at the periphery of the cytoplasm to increase the amount of extracellular cement were prevented from leaving the cell and retained within this collar, the staining capacity of which gradually increases, while the more central protoplasm, the endoplasm, becomes clearer and paler This head collar has been described in the embryonic pillar cells by N Van der Stricht as 'plaque cuticulaire,' in the adult organ by Schwalbe ('87) as 'ellipsoider Einschlusskorper,' by Joseph (00) and v Spee ('01) as 'Kopfeinschluss,' and by Held ('02) as 'Kopfkörper' 3) A change in the direction of the phalanx process and the intracephalic rootlets of its fibrils Previously (fig 1) inclined almost vertically, this fibrillar bundle gradually takes a more oblique course (fig 3, *oph*), becoming in time nearly horizontal (figs 4 and 6, *oph*) not only outside the head but also within it, the fibrils occupying its superficial part This alteration is caused doubtless by the shortening and considerable enlargement of the head and constitutes a striking evidence that this enlargement is the result not only of a sheer expansion but also of a process of stretching of its lower parts in a more horizontal and radial direction, as if pushed upward by the stream of the elongating pillar body At the same time this process involves a conspicuous shortening of the previous cephalic segment The peculiar change in the direction of the phalanx process has been observed by N Van der Stricht and by Held ('09)

Inner pillars In the adult organ of Corti the superficial portion of the inner pillar can be divided into three parts

The apex, or 'Kopfplatte' of Held the 'Innenpfeilerzellenschnabel' of v Spee and Kolmer (09) the plaque céphalique ou membrane fibrillaire' of N Van der Stricht This is a very thin quadrilateral membrane (fig 13[1]) elongated radially and stretched between the apices of the sustentacular cells (originally the outer pillars) and the sensory cells (*oh*[1]) of the first outer row and the apices of the supporting (*is*[1]) and acoustic (*ih*) elements of the inner row of hair cells It constitutes a part of

the membrana reticularis and is fibrillar in structure, the fibrils running parallel to the axis of the plate and in continuity with those of the head

The so-called 'head' is formed of at least two segments, the smaller superficial one being in close contact with the head of the outer pillar In the bat the upper part appears to be reduced from compression between neighboring elements, to a simple fibrillated lamella (fig 13[III-V] *thd*), while in the lower part there is a thin cytoplasmic layer lateral to the fibrils (fig 13[V] *thd*) In the white rat (fig 17, *thd*), the common rat (fig 18 *thd*), and particularly in the cat (fig 9, *thd*) twelve days after birth this superficial lamella is obviously thicker its lateral cytoplasmic layer being larger In the bat (fig 13[V] *thd*) and other mammals this layer increases in breadth at the level of the lower part of the head whence without any demarcation it *blends with a larger deeper segment This is not connected* with the outer pillar but is situated below the head of the latter It is a little shorter than the superficial segment and gradually tapers and continues with the body (*ipb*) of the pillar

During the first stage of its development the future head of the inner pillar is a four-sided somewhat flattened prism (figs 1, 2 and 3 *ip*) nearly uniform in diameter although tapering to its apex It is composed of a granular or homogeneous cytoplasm and a bundle of fibrils, which occupy the medial side of the lower part of the prism and the central area of its superficial portion where, in the earliest stages of development, the fibrils are arranged in the form of a hollow tubule (fig 3 *ip*) which later gives rise to a solid bundle During the second stage of development the future head undergoes no very marked changes By compression from the outer pillar head its superficial segment becomes somewhat thinner—lamellar in shape (figs 4 and 6 *ip*)—while its lower segment maintains its previous size or enlarges slightly in the neighborhood of the pillar body At the same time the transformations above mentioned are occurring in its cytoplasm in the proximity of the obturator septa In order to clearly recognize the lamellar shape of the superficial segment of the head, cross-sections are needed A longitudinal fibrilla-

tion as illustrated in figures 7 and 8 (*ihd*) indicates an oblique or more or less longitudinal section of the pillar, and such preparations are liable to misinterpretation

The most remarkable changes occur at the level of the free apices of the inner pillars the summit of the pillar scaffolding The gradual development of the head of the outer pillar, situated just beneath this summit, produces a radial extension of the latter, and the transformation of a very small square field (figs 1 2, and 3, *aip*) into a long narrow fibrillated membrane or head-plate This gradual extension is clearly shown in figures 4 (*aip*) 7 (*ipl*) and 8 (*ipl*^{i}, *ipl*^{ii}), whereas no enlargement in a spiral direction is noticeable On measuring the radial diameters of the fibrillated head plates in figures 3, 4, 7, and 8, and comparing them with the radial diameters of those portions of the membrana reticularis included between the plates and the outer border of the apices of the third row of acoustic elements, it is found that the former are respectively represented by about 1/11 1/2 75, 1/2, and 1/1 64 of the latter Thus statement gives a rather accurate picture of the rapid enlargement of the head of the outer pillar and the subsequent extension of the superficial inner pillar plate, that is, of the portion of the membrana reticularis formed by the latter during the development of the tunnel space

From this description it is also evident, according to N Van der Stricht (p 610), that the extension of the apex of the inner pillar is due solely to a mechanical factor a compression by the underlying enlarging head of the outer pillar Thus view has been corroborated by Held ('09) He does not mention the description given by N Van der Stricht but states (p 212) "Je mehr der Kopf des Aussenpfeilers sich bildet und in seiner Masse wachst, um so dünner wird über ihm die Kopfplatte des Innenpfeilers" In its extension the head plate undergoes important structural changes Originally formed of a clear cytoplasmic field (figs 1 and 3 *aip*) containing a diplosome or two central corpuscles the elongating plate becomes subdivided into two zones a lateral small clear zone enclosing the diplosome (fig 7), and a medial more extensive fibrillated one This continues to lengthen and is composed of several parallel hori-

horizontal bundle (oph^a) and is outlined by a thinner apical border or wall, and a larger basal border, the bulk of the head On penetrating into the head the fibrils of the pillar body *(oph)* become divided into two fascicuh, a thinner apical, and a broader basal one The former seems to be shorter and its fibrils spread out obliquely through the corresponding lip, the latter is longer and its fibrils spread out fanlike *(fig 15, ohd) through* the basal portion of the head, and seem to encroach upon the more homogeneous head roof When the two systems of fibrils are not stained the head roof can be more clearly seen to continue into the two borders of the notch In the cochlea of young animals *(fig 10, ohd)* the groove is much larger and its lips may be mistaken for sections through two different separate bodies, the 'ellipsoider Einschlusskorper' of Schwalbe and Joseph These bodies do exist in earlier stages of development, but later, with the roof, they form one structure—the head cap

The elongation of the phalanx process of the outer pillar will be dealt with in the next chapter

The development of the spaces of Nuel

With the exception of very short references such as those alluded to above no investigations have been carried out to determine the formation of the spaces of Nuel Hence the problem appears to be a very knotty one and almost insolvible

In the cochlea of adult animals the largest of these spaces is represented by a spiral cleft between the outer pillars and the cell bodies of the hair and supporting cells of the first outer row This space may be termed the first space or the first spiral interstice of Nuel Another cleft, which may attain considerable size, is the fourth space or spiral interstice of Nuel This contains the phalanx processes of the cells of Deiters of the third row and is included between the hair cells of the third row and the so-called cells of Hensen It is the 'external tunnel of Held (02) A second and a third space or spiral interstice of Nuel contain the phalanx processes of the cells of Deiters of the first and second rows respectively, the

former situated between the outer acoustic elements of the first and second rows, the latter between those of the second and third rows The second and third spaces do not extend between the long subjacent cell bodies of the supporting elements

Appearance of the first spiral space of Nuel This doubtless develops before the others and before any trace of the tunnel of Corti The first trace of its appearance may be seen rarely (fig 1) before the enlargement of the future heads of the outer pillars, in the form of clear, vacuolated, prominent vesicles on the lateral surfaces of the outer rods of Corti How these vesicles are produced is uncertain, they seem to be only transitory and appear rather abruptly, as though due to pressure within the clear fluid contained in the vacuolated medial zone of the outer pillars, and as though part of this fluid had been driven across the outer fibrillated zone of the cell to give rise to large prominent vacuoles These are seen along the lateral surfaces of the intermediate, the basal and occasionally even parts of the superficial portions of the outer pillars In more advanced stages their outlines and connections with the secreting cells become indistinct, and the vesicles are replaced by a common fluid mass, pervaded by a few delicate trabeculae in process of disintegration or liquefaction (fig 3, *SN*) This process is not unlike that of cytolysis by which the fluid of the tunnel is produced It is noteworthy that a distinct outline or a superficial membrane is never seen, either on the lateral surface of the outer pillars or on the medial surface of the inner pillars so that under special conditions of intracellular pressure, fluid may exude and pass into intercellular channels The cleft, filled up with this fluid, is the first space of Nuel It enlarges gradually and extends toward the membrana basilaris from which even in the adult cochlea it is separated by the lateral expansions of the feet of the outer pillars

From this description it would appear that the initial dominant factor in the development of the cleft corresponds to a difference in pressure in two parts of the outer pillars the large vacuolated medial zone where clear fluid is being accumulated,

and the surface of the fibrillated zone, where a peculiar structure, the absence of a membrane, and a lower pressure, promote an exudation of fluid In this respect a second important factor deserves due consideration i e , the shifting of the outer pillars The e structures originally are incorporated within the first row of outer hair cells and although their extremities remain always fixed, their bodies are pushed inward and inside of the acoustic elements so that at least a virtual, if not a true space appears below the first row of outer hair cells, between the nucleated portions of the cells of Deiters of the first row (fig 1, d^1) and the outer pillars (*op*) This virtual cleft contains a spiral bundle of nerve fibers and represents the future space of Nuel

The enlargement of the space of Nuel is doubtless promoted by a third peculiarity a change in the shape of the outer pillar When the shifting of the is latter completed, and before any appearance of a cleft, the lateral surface of the rod of Corti is a plane represented in a vertical or oblique section by a straight line Along with the lateral extension of the foot upon the basilar membrane (fig 3, *op*) and the appearance of the head (figs 4 and 7 *ohd*) and by a considerable elongation of the intermediate portion (the future body, fig 7, *opb*), which is only possible by virtue of a curvation the straight line becomes markedly curved its concavity being turned towards and embracing the cleft This may be a more important factor than appears at first sight Indeed, in previous investigations (in press) it has been noted that the shifting of the cells of Deiters may be completed (that is to say the sustentacular elements of the first outer row may be situated beneath their corresponding hair cells) before any appearance of a tunnel space (fig 9 of the previous paper), or even of the true space of Nuel In such figures the original straight line persists although the heads of the outer pillars are large but the lateral extension of the feet is delayed

Structure and transformation undergone by the phalanx processes of the outer pillars Should any doubt be entertained as to the process of cytolysis along the lateral lower parts of the outer pillars, the structures and the transformation undergone by

their phalanx processes afford striking evidence of such a liquefaction In the above description of these apical bands which unite the phalanges to the outer pillar heads, the most distinct constituent, the fibrillated bundle, alone has been mentioned In the early stages of the development the band is composed of fibrils collected into a fasciculus, which is surrounded by a clear granular cytoplasm Before the space of Nuel reaches the membrana reticularis this phalanx process proper is very short, being limited to the portion running between two neighboring hair cells (figs 4, *oph* 8 and 11, oph^{II}), and the portion lying under the phalanx itself (figs 8 and 11, oph^{II}) In other words, the enlarged head contains the longest part of the fibrillar bundle (figs 8, oph^{IV}, fig 11, oph^{III}, oph^{IV}) and covers completely the head plate of the inner pillar The roof of the developing space of Nuel is made up of two strata, the lateral thinnest part of the outer pillar head (fig 11, oph^{III}, compare with figs 6 and 12), and the lateral part of the superficial striated membrane (fig 8, ipl^{II}) When the first interstice of Nuel has attained its entire extent in the adult organ its roof is composed of the lateral part of the head plates of the inner pillars (figs 13[1] 17, and 18, ipl^{II}) strengthened by equidistant parallel, fibrillated bundles, portions of the ultimate phalanx processes (figs 13^{III}, 17, and 18, oph^{III}), which run in an oblique direction toward the spiral rows of pillars (fig 13^{II})

Figures 13^{I}, 13^{II}, and 13^{III} illustrate the structures of this roof at these successive levels in the adult organ of Corti Between the apices of the inner hair cells (*ih*) and the outer sensory elements (oh^{I}) they show respectively a superficial plane—the striated head-plates of the inner pillar cells (fig 13^{I}, ipl^{II})—an intermediate plane composed of parts of the preceding plates (fig 13^{II} ipl^{I}) and parts of oblique subjacent fibrillar bundles, and a deeper plane (fig 13^{I}) showing from the axial to the lateral side the row of fibrillated lamellae, heads of the inner pillars (*ihd*) the row of outer heads, a gap nearly as large as the preceding row and bridged across by equidistant fibrillar bundles (oph^{III}), entirely devoid of clear cytoplasm The gap is the upper floor of the space of Nuel (SN) which is

of Deiters of the third row, which remain in situ between the third row of sensory elements and the cells of Hensen, will appear the large fourth interstice

Before the appearance of any space the phalanx process is composed of a clear cytoplasmic sheath, enclosing a darker, axial mitochondrial strand, which by juxtaposition and fusion of the chondrioconts becomes gradually transformed into an axial fibrillated filament The process is larger at its base, which issues from the nucleated cell body and tapers to the superficial membrana reticularis

In a kitten nine days old, at the level of the apical spiral turn of the cochlea the second, third, and fourth sustentacular interstices are still filled up with the unmodified phalanx processes so that intercellular spaces are absent In the second turn, narrow channels appear and are somewhat larger near the surface of the epithelium than towards the base of the processes Inversely the processes have become reduced in diameter at the expense of their clear protoplasm At the level of the basal or third turn the enlargement of the spaces of Nuel and the reduction in size of the cytoplasmic sheath of the phalanx processes are much more marked It must be noted that the thinning out of the latter is not the result of a sheer concomitant elongation, for these alterations are accompanied by a considerable elongation of the nucleated cell bodies of the sustentacular elements, involving a subsequent shortening of the supported hair cells, hence of the neighboring phalanx processes These become more slender on account of a process of elaboration and secretion and a subsequent extrusion of clear fluid from the protoplasmic sheath Whether, as many preparations seem to prove, this discharge is accompanied by a process of true cytolysis is uncertain, for these structures are very delicate and the shrinkage caused by the reagents might give rise to artefacts liable to misinterpretation

The fourth space of Nuel develops in the same manner as the second and third, and when it appears, is but little larger than the others It is occupied by the apical processes of the cells of Deiters of the third row Originally as long as the neigh-

boring sensory elements, these processes are more numerous (previous paper) and larger than those of the first and second supporting rows Situated outside the hair cells of the third row, they are not squeezed and impeded in their lateral expansion like the others It is not to be wondered at that the products of secretion or cytoplasmic disintegration around the apical fibrillated bundles are more abundant and result in an expansion of the fourth interstice Nevertheless, the process of development is identical with that of the two preceding spaces and therefore the application of a special term, external tunnel to designate this formation is unnecessary However, there can be no doubt that phalanx processes of the cells of Deiters of the third row retain their cytoplasmic sheath much longer than the others, and may show parts of it in the adult cochlea as pointed out by Held ('02) for the 'apical type' of these cells in guinea-pig cat, dog, and even the mouse In such cases these processes are in closer connection with the outer wall of the space than with the medial

In the basal spiral turn of the cochlea of a kitten twelve days after birth (fig 12), the floor of the second third and fourth spaces of Nuel is formed by parts of segments of the cells of Deiters (d^{I}, d^{II}, d^{III}) supporting their corresponding sensory elements (oh^{I}, oh^{II} oh^{III}) The medial and lateral boundaries of the second interstice are represented, respectively by the lateral surfaces of the hair cells of the first row and the medial surfaces of those of the second The medial and lateral boundaries of the third interstice are represented respectively by the lateral surfaces of the hair cells of the second row and the medial surfaces of those of the third The adjoining surfaces of the acoustic elements of each sensory row are separated by narrow clefts through which all of the spaces of Nuel intercommunicate These channels originally occupied by the phalanx processes of the sustentacular cells (those of the first sensory row being the superficial segments of the outer pillars) are liberated after the shifting of the phalanx processes At first very narrow and virtually obliterated by the process of enlargement of the hair cells these intercellular clefts become wider by the reduction in

11 The second, third, and fourth spaces of Nuel are located, respectively, between the first and second, the second and third, and the third row of hair cells and the cells of Hensen (atrophied hair cells of a fourth row) These spaces do not extend down between the sustentacular elements, but communicate with each other and with the first space through clefts between the hair cells These intercellular channels, originally occupied by the phalanx processes of the sustentacular elements, become free after the shifting of the latter into the neighboring medial spaces the phalanx processes of the cells of Deiters of the third row remaining in situ

12 Each of these phalanx processes is composed of an axial, fibrillar filament and a peripheral, clear, cytoplasmic sheath In the course of development this sheath becomes thinner and may disappear by a process of secretion, which gives rise to the fluid contents of the primitive second, third and fourth spaces of Nuel

13 The roofs of the second third, and fourth spaces of Nuel and of the intercellular clefts between two neighboring hair cells of each sensory row are made up of delicate membranes, partially fibrillated which belong to various parts of the phalanges of the sustentacular elements

14 The fluid contents of the tunnel and the first space of Nuel are separated from the fibrillated basement membrane of the membrana basilaris by a thin protoplasmic covering belonging to the feet of the inner and outer pillar cells They intercommunicate through clefts between the outer pillars and communicate with those of the second, third, and fourth spaces of Nuel The fluid of all the spaces of Nuel is separated from the endolymph of the cochlea duct by the roofs of these interstices, very thin membranes, entirely or partially fibrillated Such structures doubtless promote the propagation of vibratory waves from the basilar membrane to the membrana tectoria, contained in the cochlear canal

All the material and reagents necessary for the present investigations were supplied by Dr T Wingate Todd, Director of the

Anatomical Laboratory of the Medical School, Western Reserve University, Cleveland, Ohio It affords the author great pleasure to express his deep gratitude to Dr Todd

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PLATE 1

EXPLANATION OF FIGURES

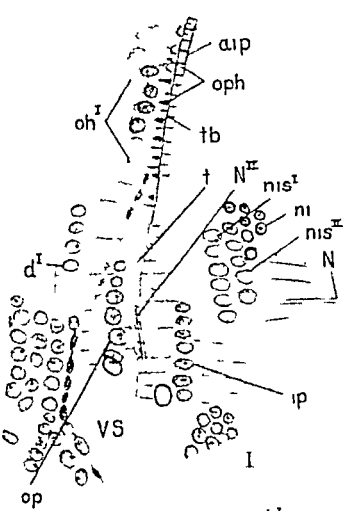
1 Section tangential (and somewhat oblique) to the surface of the organ of Corti through the second (middle) turn of the cochlea New-born kitten Fixation osmic acid, 1 per cent aqueous solution for about one hour followed by immersion in Zenker's fluid Stain Iron hematoxylin, Congo red, light green

2 Section tangential to the surface of the organ of Corti through the second turn of the cochlea Kitten 3 days, 12 hours after birth Exposure of the cochlea, the bony wall of which had previously been provided with two small openings, to vapors from a 2 per cent aqueous solution of osmic acid for approximately one hour, and subsequent treatment of the piece by trichloracetic acid 5 per cent in water Iron hematoxylin, Congo red

3 Section tangential to the surface of the organ of Corti through the basal portion of the second turn of the cochlea Dog 3 days, 18 hours after birth Zenker's fluid Iron hematoxylin, Congo red

4 and 5 Sections tangential to the surface of the organ of Corti through the basal portion of the second turn of the cochlea Kitten 3 days after birth Solution of trichloracetic acid 5 per cent in water Iron hematoxylin, Congo red light green

6 Radial vertical section of the organ of Corti through the third (basal) turn of the cochlea Kitten 3 days, 12 hours after birth Exposure of the cochlea to vapors from a 2 per cent aqueous solution of osmic acid and subsequent treatment of the piece by trichloracetic acid 5 per cent in water Iron hematoxylin, Congo red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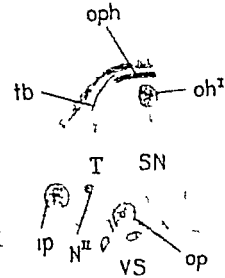
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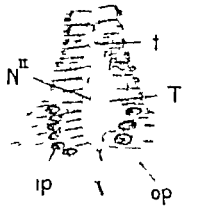
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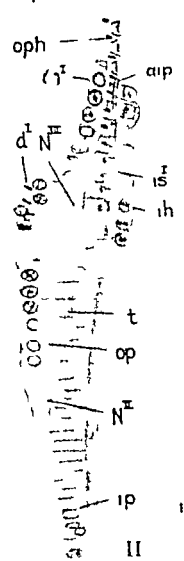
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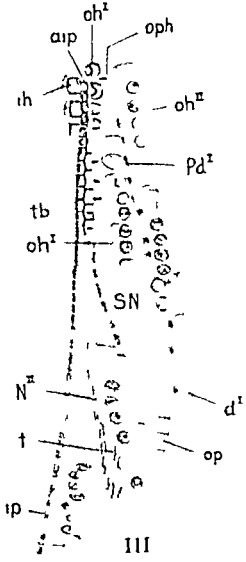
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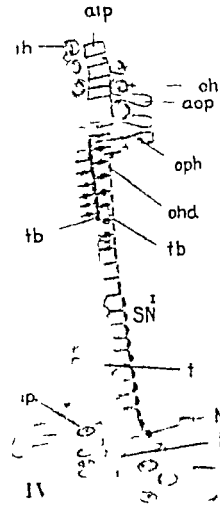
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PLATE 8

EXPLANATION OF FIGURES

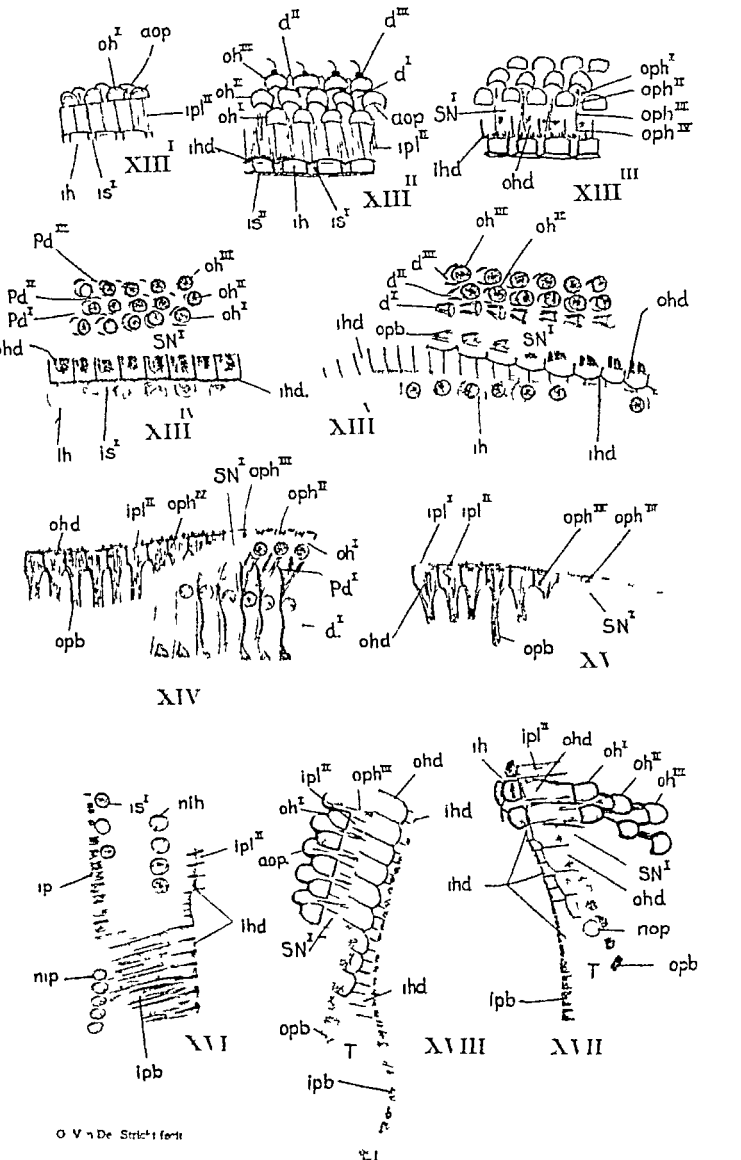
12–14 Sections tangential to the surface of the organ of Corti through the third turn of the cochlea. Adult bat (Vespertilio fuscus). Zenker's fluid. Iron hematoxylin. Congo red. The figures illustrate structures at five successive levels of the organ of Corti.

11 and 15 Vertical spiral sections of the organ of Corti through the second turn of the cochlea. Adult bat (Pipistrellus subflavus). Bouin's fluid. Iron hematoxylin. Congo red, light green.

16 Vertical spiral section of the organ of Corti through the second turn of the cochlea. Adult bat (Pipistrellus subflavus). Trichloracetic acid. Iron hematoxylin. Congo red, light green.

17 Section tangential to the surface of the organ of Corti through the second turn of the cochlea. Adult white rat. Trichloracetic acid. Iron hematoxylin. Congo red, light green.

18 Section tangential to the surface of the organ of Corti through the third turn of the cochlea. Adult rat (Mus decumanus). Bouin's fluid. Iron hematoxylin. Congo red, light green.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Resumido por el autor, Howard Ayers

Cefalogénesis de los Vertebrados

IV Transformación del extremo anterior de la cabeza, resultante en la formación de la "nariz"

El presente trabajo trata de la filogénesis de la nariz de los vertebrados y se basa en el estudio detallado del extremo anterior de la cabeza en Amphioxus, Ammocoetes, Petromyzon, Bdellostoma y el chimpancé, junto con consideraciones sobre descubrimientos previos por varios anatómicos con relación a los nervios terminal y vómero-nasal. El tabique nasal puede considerarse como un antiguo jalón en la anatomía de los vertebrados. Reconocemos en el extremo anterior de la cabeza del Amphioxus la condición más primitiva de esta estructura en las formas vivientes. Se transforma en el tabique nasal de los vertebrados después que se aloja en él el bulbo trigémino-nasal. Son muchas las variaciones en las dimensiones del tabique y en el tamaño y disposición de las cámaras nasales, pero la morfología fundamental permanece intacta. El hecho de que los nervios terminal, olfatorio y septales también existen en los marsipobranquios estudiados por el autor, junto con los hechos previamente establecidos en otros vertebrados, incluso el hombre, demuestran que los tres pares de nervios craneales están incluidos en la estructura septal y, por consiguiente, están alojados en la cámara nasal. El número de nervios craneales en el hombre es por esta causa, catorce, no doce. Los órganos sensoriales inervados por los tres nervios craneales pertenecientes exclusivamente a la cámara nasal son órganos del sentido químico y funcionan como órganos destinados a reconocer los alimentos respiratorios y alimenticios. La invasión del territorio periférico terminal de los nervios craneales sensitivos por parte de nuevos elementos de origen anatómico diverso y, a lo más, su alianza funcional ulterior, es tal vez un caso único. Está demostrada por la distribución de las ramas sensoriales del trigémino en la cubierta epitelial del tabique, que originariamente estaba inervado exclusivamente por los nervios de los sentidos químicos.

Translation by José F. Nonidez
Columbia University

VERTEBRATE CEPHALOGENESIS

IV TRANSFORMATION OF THE ANTERIOR END OF THE HEAD, RESULTING IN THE FORMATION OF THE 'NOSE'

HOWARD AYERS

TWENTY-SIX FIGURES

CONTENTS

INTRODUCTORY

From the investigations of many anatomists we have come to recognize, first, that the jaw apparatus (including the tongue and mouth) is a mechanism built up out of old-time head cartilages and newer elements derived from the gills, which has been added to the primitive vertebrate head—in fact a mechanism of the trigeminus Second, that the whole auditory apparatus is a mechanism built up out of surface sense organs sunk below the surface, together with structures derived from the gills Third, that the eyes and adnexa are the end result of the outpushing from the brain of two hollow globes, whose walls are made up of the pigmented light sensitive cells of that part of the central nervous axis behind the lamina terminalis, with numerous associated parts These three prominent organ systems have been quite thoroughly worked out and their component parts traced back to their origins In speaking thus of the evolution of these three prominent additions to the head, we include those other necessary structures, as blood, nerve and lymph supply, and the muscles connective-tissue parts and skin, which go to correlate

and cover these organ systems As regards the nose it is generally agreed that the olfactive organs are derived from a pair of sensory organs formed near the anterior end of the head which very early sink below the surface of the skin of the snout later to become housed in a cavity called the nasal chamber. The details of this process and the structures involved have not been definitely worked out and described

The following condensed account of some of the results of my study of the vertebrate nose has for its object making clear the manner in which the human nose has come to be and also to homologize the several structures entering into its formation The literature is extensive and regarding such structures as e.g Jacobson s organ the N terminalis. and the vomeronasal nerve by no means harmonious either in statement of fact or interpretation No attempt will be made here to review the literature it being considered more important to lay the foundation for a rationals tudy of the nose and nasal region of the vertebrate head.

Beginning with the stage of head development presented by Amphioxus we pass to Ammocoetes Petromyzon Bdellostoma and Man Already in the Marsipobranchs the main features of human nasal anatomy are laid down since the nasal chamber of Bdellostoma contains the terminal organs supplied by three pairs of cranial nerves with the endings of the invading branches of the trigeminus

1 AMPHIOXUS

The anterior end of the head region of Amphioxus (figs. 1 and 12) is compressed from side to side and has the shape of a spear-head viewed either from the side or above. Included within it we note the anterior end of the central nervous system with the terminal paired but unseparated eye rudiments and the primitive olfactive organs. This part of the nervous axis contains the ventricular cavity and is the earliest stage known to us of the vertebrate brain Connected with the brain from before backward are the following paired nerves (figs. 1 2 3 4 5 6 7 and 12) 1 N terminalis 2 The N opticus which does not extend beyond the brain contour 3 N olfactorius. 4 N septalis.

The N terminalis[1] is connected to the right and left side of the anterior end of the ventral plate of the brain, and owing to the relatively large size of this pair of nerves they appear, when viewed from above, like a bifurcation of the brain They run forward to the tip of the head The N opticus is entirely imbedded in the front wall of the brain anterior to the ventricle In Amphioxus we have a stage of the evolution of the eye antedating the formation of optic cups which, from Bdellostoma on, presents such a prominent feature of vertebrate anatomy The N olfactorius (figs 2, 3, 4, and 7), being relatively small, is a short nerve which arises from the anterodorsal wall of the brain near to the median line and, while the right and left olfactorius arise from the right and left halves of the brain respectively, they are usually drawn close together into one trunk as they approach the olfactive organ, although they occasionally remain separate and distinct their entire length They run dorsad and cephalad and innervate the right and left halves of the olfactive cup just as their homologues do in Ammocoetes and all other vertebrates The N septalis arises from the dorsal wall of the brain on either side of the median line above the posterior limit of the ventricular cavity The two nerves curve upward, forward, and outward, and run to the sides of the anterior end of the head, innervating the territory mainly caudad of the N terminalis and as far back as the hypophysis

In Amphioxus the body surfaces innervated by the terminal and septal nerves (figs 1 and 12) are fully exposed, except the hypophysial region which lies within the buccal cavity, with this exception they form part of the body contour The olfactory organs are sunk below the body surface as a conical pit opening directly on the surface and more or less pushed to the left of the median line by the dorsal head fin fold[2]

The distribution of the septalis nerve in Amphioxus is as follows Arising from the dorsolateral territory of the brain above the posterior border of the ventricle the nerve trunk soon separates (figs 1, 12 and 26) into two parts The larger part curves forward and outward, dividing as it passes to its terminal territory behind the tip of the snout which is supplied by the N

terminalis The smaller branch curves forward and downward over the surface of the notochord and innervates the surface territory about the anterior end of the buccal cavity as well as the walls of the terminal pocket of the mouth and the dorsal hypophysial organ which formerly occupied a surface position as the preoral pit of the larva

The terminal territory of the Amphioxus body is thus a terminal sensory organ As far as known, the sensory elements are isolated sensory cells distributed with some regularity of spacing throughout the epithelial covering of the body in the territory supplied by the terminal and septal nerves These sensory cells have not been found in other localities They are innervated by terminal twigs given off from the short fibrils which issue from the groups of subepithelial ganglion cells belonging to these two nerves (fig 11) The close association of the terminal and septal nerves in their peripheral distribution is reflected in the central exchange of fibers (figs 8, 9, and 10) and doubtless in their physiological functions, to the extent of their being grouped to-

ABBREVIATIONS

A, place of origin of the hypophysial organ
B, last surface position of hypophysial organ
Br, brain
C, position of hypophysial organ in adult
G, nasal gland (Jacobson's) organ
H, hypophysis
HC, hypophysial canal
h, hypophysial extension
H S , hypophysial sac
L O, lobus olfactorius
l t, lamina terminalis
M, mouth
M', anterior buccal pouch
N, nose
n, external nasal opening
n f, nasal fold
n n, nasal nerve
NC, nasal canal
NF, nasal fold
N p, nasopalatine nerve
O, olfactory nerve
OL, *L O*, olfactory lobe
ON, olfactory nerve
op, optic nerve
P, anterior buccal pouch
PN, prenasal sac
SN, septalis
Sk, cranial wall
Sp, nasal septum
S, hypophysial branch of septalis
T, *n* terminalis
T &S, n terminalis and n septalis
TO, terminal organ
TH, hypophysial branch of terminal nerve
tt, terminal nerve bundle
V, velum
v, valve
Vt, ventricle

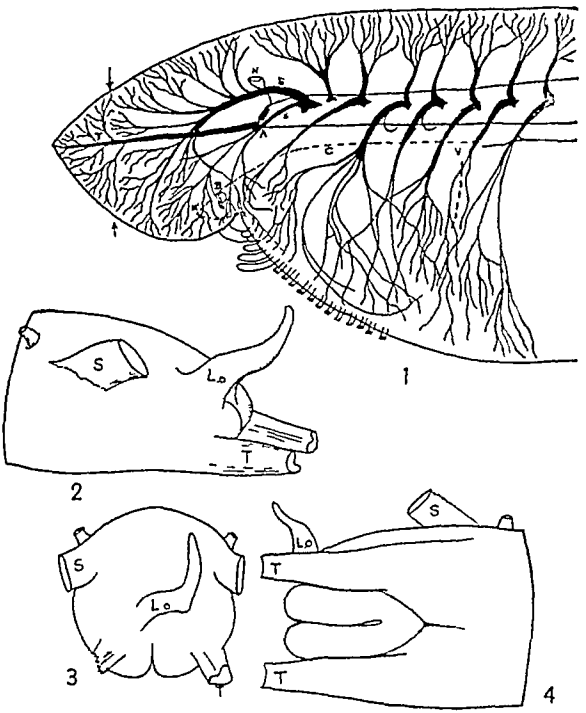


Fig 1 View of left side of the head of Amphioxus showing brain and nerves The terminalis opticus olfactorius , and septalis are the first four cranial nerves The apical territory supplied by the terminal and eptal nerves is distinctly marked off from the rest of the head and its close association with the optic and olfactory sensory structures is evident The septal territory has already undergone partial migration into the buccal cavity The path of migration of the hypophysial organ is indicated by the letters *A* and *B* and the dotted line *B-C* Over the outline of the apical buccal pouch is shown the last surface position (in the larva) of the pre-oral pit (hypophysial organ) before its entrance into the mouth chamber

Fig 2 Anterolateral view of the brain of Amphioxus (short type) showing the terminal septal and olfactory nerves and the region of the lamina terminalis

Fig 3 Anterior view of the same brain of Amphioxus

Fig 4 Ventrolateral view of the same brain of Amphioxus showing the first three nerves and the infundibular territory

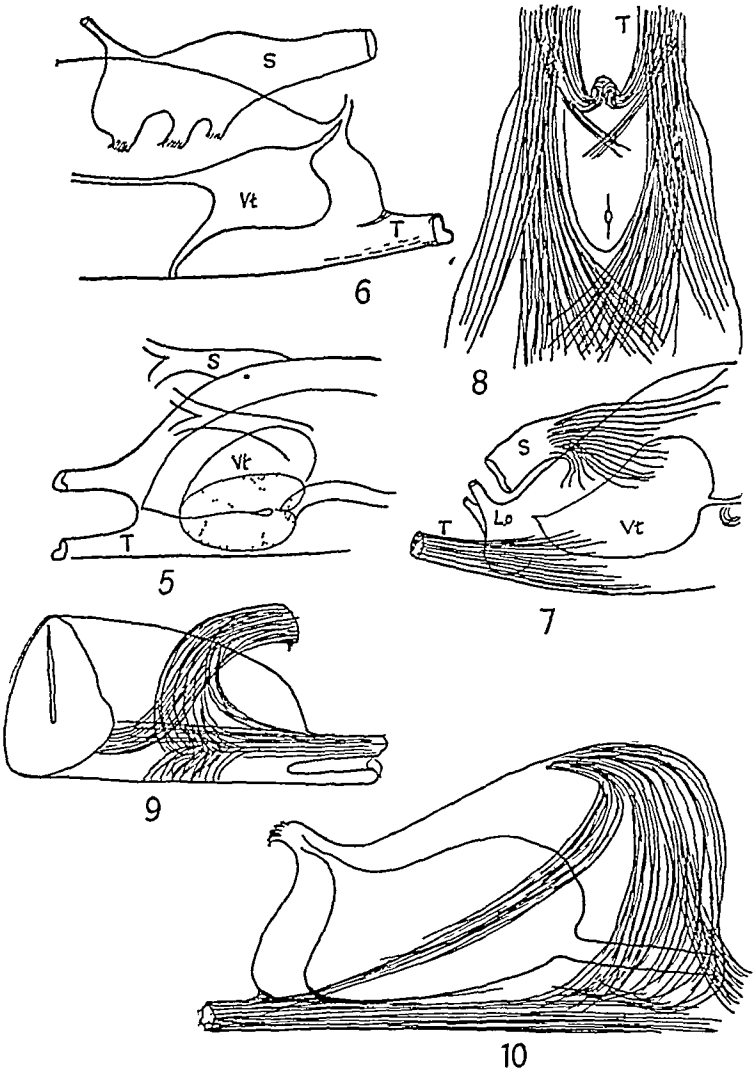


Fig 5 Anterodorsolateral view of a brain of Amphioxus (transparent), to show the ventral territory of the neuropore in the adult

Fig 6 Lateral view of the brain of Amphioxus, to show the roots of the septal nerve, the ventricle with its olfactory, infundibular, and spinal prolongations

Fig 7 Dorsolateral view of brain of Amphioxus, to show paired olfactory nerves

Fig 8 Ventral view of brain of Amphioxus to show the course of fibers of terminal nerve

Fig 9 Posterior ventrolateral view of Amphioxus brain, to show course of fibers of terminal and septal nerves

Fig 10 Anterolateral view of another brain, to show course of fibers of terminal and septal nerves

gether as a single nerve physiologically The remarkable persistence of these two nerves as anatomically distinct structures throughout the entire vertebrate series from Amphioxus to man is noteworthy and needs further attention from anatomists The concentration of the chemical sense organs, olfactive sense organs, and light perceptive organs at the anterior end of the neural axis is strikingly shown in Amphioxus and the anatomy of the nasal chamber of man as herein described shows that the morphological relations of these structures have not been much disturbed throughout the evolution of the vertebrates In Amphioxus the neural lips of the anterior neuropore maintain their original relations in the adult This is indicated by the position of the terminal and septal nerves which supply the tip of the head and the organs connected with the ventral end of the neuropore, the hypophysis, while the olfactory nerves supply the organs connected with the dorsal end of the neuropore, the nose The whole neuroporic territory is concerned with testing alimentary and respiratory supplies, i e , food in the broad sense

2 AMMOCOETES AND PETROMYZON

The head region of Ammocoetes has the shape of a truncated cone with the snout truncated from above, downward, and backward In the larval Petromyzon the superficial territory innervated by the terminalis and septalis which in Amphioxus forms part of the body surface is withdrawn into the protection of a nasal canal along with the olfactory organ and opens secondarily to the outside through the nasal canal Confining our attention for the present to the morphological equivalents of the parts just described in Amphioxus, we find the spear shaped primitive tip of the head withdrawn bodily into the nasal chamber to form the nasal septum of Ammocoetes each half receiving a rich nerve supply from its N terminalis (fig 13) The characteristic condition of the septum in Ammocoetes furnished the key for the solution of the problem of the homologies of the nasal organ of vertebrates On either side of this septum (fig 14) we find the olfactive organs each with its N olfactorius The N septalis

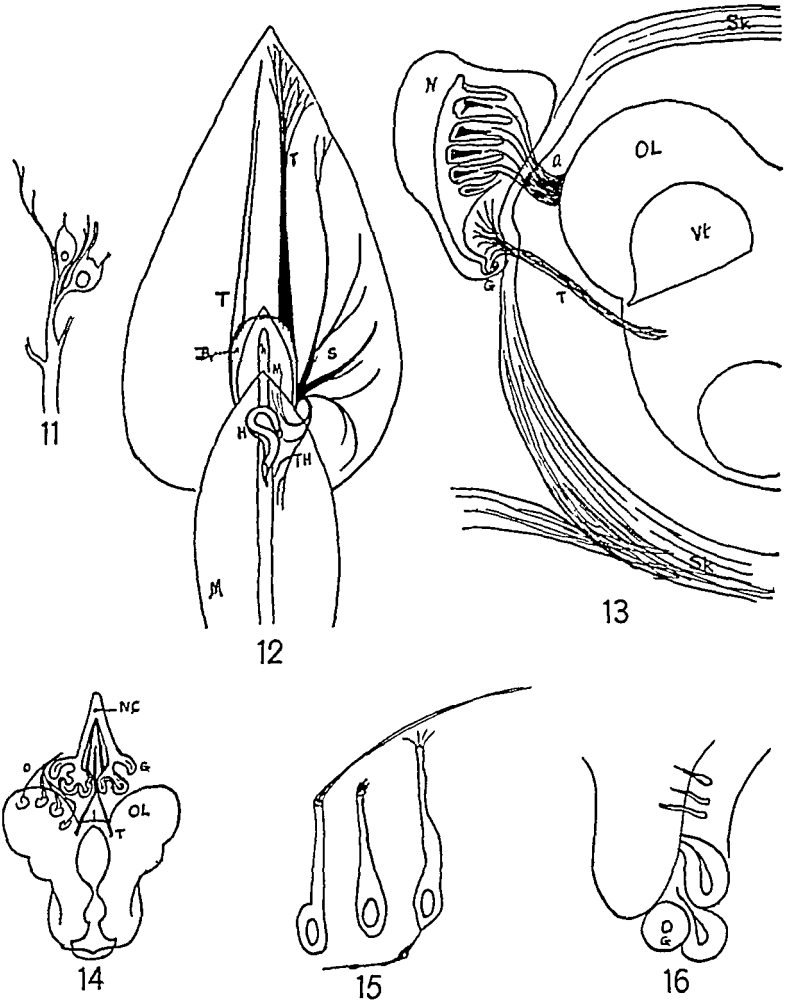


Fig 11 End twig of terminal nerve with ganglion cells and peripheral fibrils

Fig 12 Ventral view of snout and mouth of Amphioxus, to illustrate relations of brain to anterior buccal pouch, and the distribution of the terminal and septal nerves as seen from below The hypophysial organ with dorsal buccal groove running caudad and the band of thickened epithelium running cephalad are shown In the drawing the head is expanded laterally to make room to show the parts clearly

Fig 13 Sagittal section of brain and nasal organs of Ammocoetes, to show relations of olfactory and terminal nerves to the brain and the olfactory and terminal organs

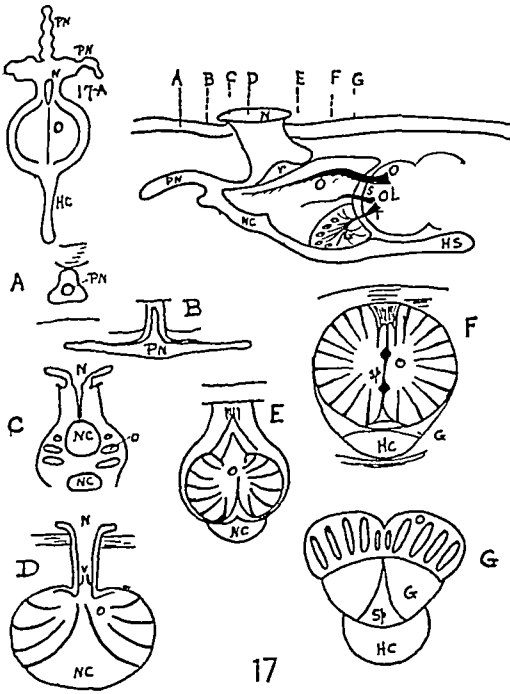
Fig 14 Horizontal section of brain and nasal organs of Ammocoetes, to show relations of olfactory and terminal nerves and the nasal septum

Fig 15 Three cells from the 'nasal' epithelium of Ammocoetes, one olfactive sense cell and two ciliated epithelium cells from the ciliated 'gland' of the terminal organ

Fig 16 Part of a section of the 'gland' of the terminal organ

passes to the septum along with the median bundles of the olfactory nerves The optic nerves are well developed and leave the base of the brain below and behind the N terminalis Owing to the formation of heavy lips surrounding the mouth—which in the adult became the 'sucking disk'—the nasal tube opens on the top of the head in front of the brain region The olfactory organs and nerves are completely separated from each other in Ammocoetes by this nasal septum, which from its origin we recognize as a fundamental as well as an original landmark in head anatomy At the base of the septum is a glandular structure (figs 14, 15, and 16) which is innervated by the terminal nerve This paired gland is a more or less constant feature of the vertebrate nose from Ammocoetes up to man It has been described in many forms as the organ of Jacobson Between the Amphioxus and Ammocoetes condition of the terminal and nasal region of the head there has occurred a translation of this region ventrad and caudad in the sagittal plane with a concomitant enlargement of the nasal organ and the separation of its right and left halves by the interposition of a septum formed by the spear-shaped tip of the head In other words, the olfactive organs have migrated ventrad along the sides of the septum Thus the olfactive organs come to lie laterad of the terminal and septal territory, instead of dorsal, as in Amphioxus

In Petromyzon marinus the tip of the primitive head has been sunk still further below the surface of the body and surrounding it the nasohypophysial canal has been complicated by the formation of sacs and pockets with valves and folds for the reception and control of the water to be tested The olfactive organs have been expanded and pushed forward as well as downward, while the terminal and septal structures occupy the ventroposterior portion of the nasal capsule This portion of the complicated nasal organ has been described as a gland It is made up of a series of pockets or tubes which open out on the face of the wedge-shaped terminal organ to become continuous with the folds of the septal region of the nasal chamber (figs 17 and 17A)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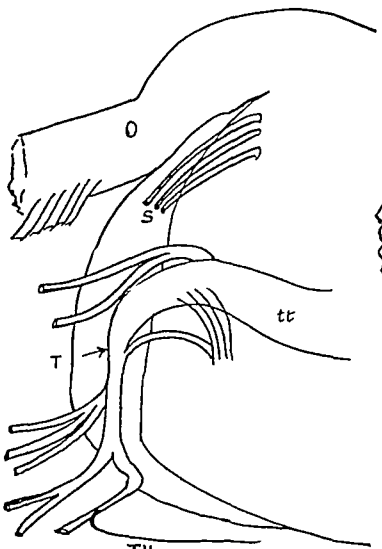
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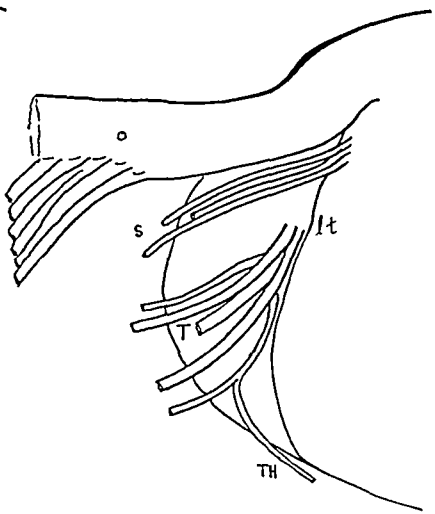
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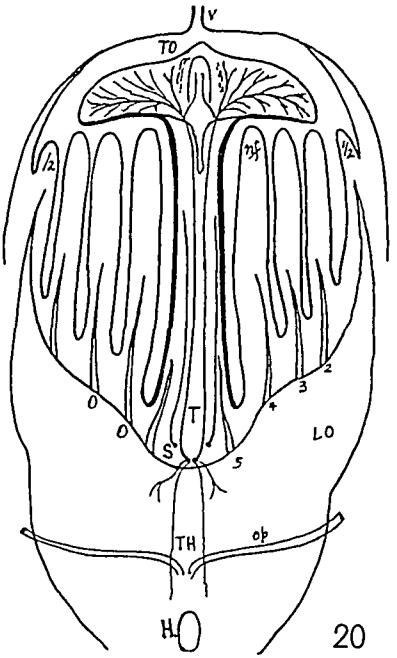
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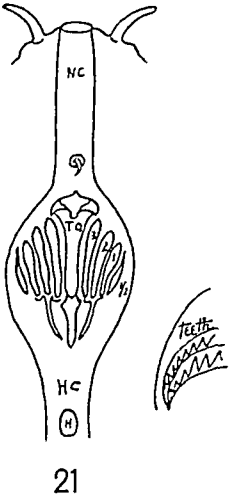
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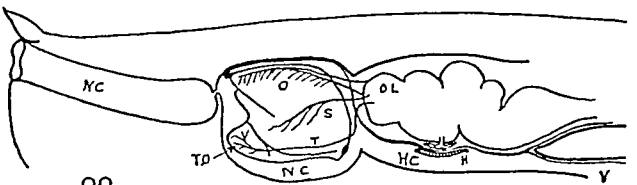
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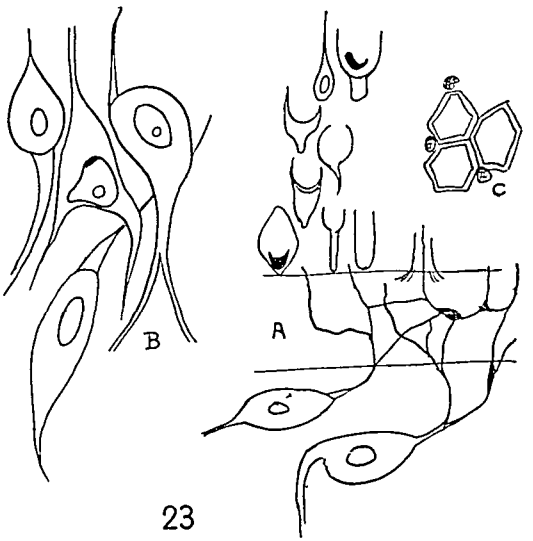
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brain inside the median fissure and near its anterior end, but separate from the median olfactory nerve bundle which leaves the tip of the median part of the olfactory lobes The septal nerve also runs ventrad before coursing forward to supply its peripheral territory The olfactory nerves are enormously developed and the nerve of each side supplies three complete and two half folds (plates) of the compound nasal organ, all of which, heretofore, has been called the olfactory organ The N terminalis in Bdellostoma presents a stage intermediate between Amphioxus and the Selachians[3] In the former, the olfactory nerves are small and in their primitive terminal position on either side of the dorsal end of the neuroporic raphé, while the N terminalis is relatively large, leaving the brain nearly midway between the olfactory organ and hypophysis, and strictly terminal in its positions as regards the adult brain

In Bdellostoma the olfactory nerves have become enormously increased and overshadow the N terminalis, which, while it remains terminal in its peripheral distribution leaves the brain near the anterior end of the olfactory lobes within the median fissure The development of the olfactory lobes is so great that the primitive anterior end of the brain is covered over and its relation obscured The great increase in size of the olfactory nerves causes them to enfold the forebrain in an inclosing overgrowth of the olfactory lobes, which apparently forces the roots of the terminalis upward, but they retain their relative position with reference to the exit of the olfactory nerve, viz , mesad and ventrad of it In Amphioxus we found branches of the septal nerve

Fig 20 Ventral view of Bdellostoma nasal organ to show the distribution of the olfactory terminal and septal nerves the median terminal organ and other nasal folds

Fig 21 Same view of nasal organ and nasohypophysial canal in Bdellostoma

Fig 22 Lateral view of nasal organ brain and nasohypophysial canal of Bdellostoma

Fig 23 A Section through the mucous covering terminal organ of Bdellostoma Five layers of epithelium cells are shown above the basement membrane beneath which lies a plexus of nerve fibrils given off from the ganglion cells lying below B Four ganglion cells from same section C Surface view of epithelium from same terminal organ to show relation of sensory cells to other surface cells

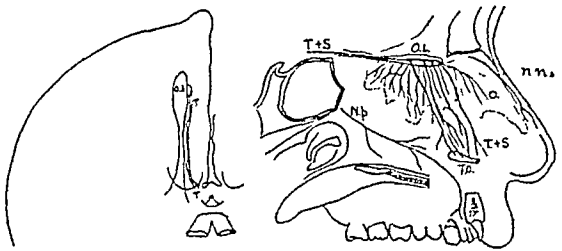
innervating the hypophysial organ In Bdellostoma the hypophysial branches are given off from the terminal nerve In mammals it has been shown by Huber and Guild[4] and Larsell[5] that both terminal and septal branches run to the organ of Jacobson, i e, the terminal organ In Bdellostoma, the ganglion cells of the terminal nerve lie in the terminal organ (fig 23)

4 CHIMPANZEE AND MAN

In man as described by Brookover[6] and in the chimpanzee, as my dissections disclose (fig 24) the N terminalis leaves the brain ventrad and mesad of the olfactorius and passes forward to the lamina cribrosa In man they pass through this plate along with the septal and olfactory nerves and run ventrad to terminate in and about Jacobson's organ In the chimpanzee the nerve on leaving the brain enters the pia and takes its course forward to near the lamina cribrosa where it passes into the dura and leaves the cranial chamber along with the bundles of olfactory fibers Its course outside the cranial chamber was not traced The recently published researches of Larsell[5] on several mammals show conclusively that both the terminal and septal nerves are, in this class, preserved in their original relations to the olfactory nerve and brain The nasal chamber in man, therefore, contains the surface distribution of these three most ancient cranial nerves as well as the surface terminations of invading branches of a fourth and more recent cranial nerve, the trigeminus (fig 25)

5 THE ANTERIOR CRANIAL NERVES

We thus find that the nasal septum and related parts form one of the most ancient and least changed morphological complexes in vertebrate anatomy Reacting to physiological necessity, the advanced outposts of the central nervous apparatus of vertebrates were withdrawn early in the life of the phylum more or less deeply into the protective hood furnished by the overgrowth of the muscles supported by added skeletal structures, and body covering from territory lying behind the region of the primitive



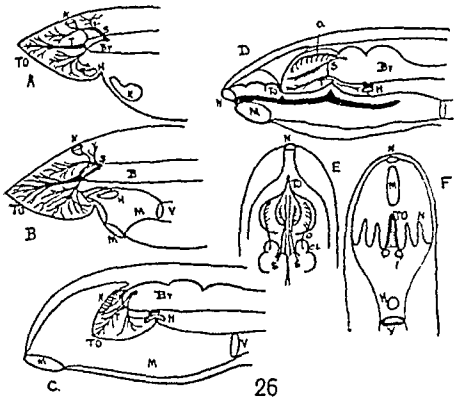


Fig 24 Ventral view of right cerebrum of Troglodytes (chimpanzee) to show intracranial course of N terminalis

Fig 25 Sagittal section of nasal chamber of man from Toldt to indicate extracranial course of N terminalis drawn in from Brookover's description The septal nerve (Vomerona al) practically parallels the course of the terminal branches The invading branches of the trigeminus are also shown

Fig 26 Six diagrams illustrative of the translation of the chemical sense organs of the neuropore territory of Amphioxus from the exposed position on the surface of the head to the inclosed condition found in Bdellostoma where they are housed in the nasal chamber A Pre Amphioxus stage with the hypophysial organ still outside of and in front of the buccal cavity B Amphioxus stage with the hypophysial organ inside the buccal cavity, said organ being the first of the apical territory to find protection C Post Amphioxus stage in which the overgrowth of the trigeminal structures has housed in the remainder of the apical territory of the Amphioxus head This apical region furnishes the nasal septum together with the sensory structures associated with it in the nose of the higher vertebrates D Bdellostoma stage in which a nasopharyngeal partition has appeared partly separating nasal and buccal chambers E Ventral view of stage C F Ventral view of stage D

brain, in fact, from the territory of the trigeminus nerve, we therefore find that the advanced outposts of the brain, the most anterior sense organs of the body surface, in all forms above Amphioxus are tactile sense organs of simple or complex structure belonging to the trigeminus as distinguished from the earlier group of chemical sense organs, belonging to the terminal, olfactory, and septal nerves which form the anterior sensory outposts in Amphioxus This change in the character of the sensory outposts is of course a result of forward overgrowth of the trigeminus mechanisms We also find that the trigeminus structure has not only surrounded and housed in this group of chemical sense organs, but has also invaded the original olfactory and terminal sensory territories supplied by the NN terminalis, olfactorius, and septalis, and here performs some function of a tactile sort Along with the housing of the chemical sense organs there has been built up in the long series of vertebrate forms a large variety of control systems of valves, doors, guide folds for the control of water and air currents, their admission, guidance, and expulsion varying in different animals as the case may be The emergence of vertebrates from water breathing to air breathing has not affected the physical conditions of the functioning of the chemical sense organs, for they still are kept wet and pick up their stimuli out of a liquid medium, thanks to the moisture supplied by the 'mucosa' With the exception of the jaw apparatus and related parts, no single change of, or addition to head structures has caused greater changes in contour or anatomical detail than the housing of the chemical sense organs Owing to the failure to recognize the presence of both the terminal and septal nerves, the first arising ventrad of the olfactorius, while the second arises dorsad of the olfactorius, much confusion exists in accounts of the so-called nervus terminalis In all vertebrates yet examined, one of these nerves is found to be present, which may arise near the dorsal surface of the brain or near the ventral surface In many forms both pairs of nerves have been found Further investigation will doubtless show that both nerves are present in all vertebrates In order to make final decision, it will be necessary to trace the

nerves to their central as well as peripheral terminations The presence of coördinating sympathetic fibers in the terminal and septal nerves seems to be definitely proved in a number of the species studied The diagrams shown in figure 26 cover four stages in the transformation of the apical head region of Amphioxus into the nasal chamber and septum of the higher forms

Stage A is pre-Amphioxus condition The hypophysial organ (pre-oral pit) is shown on the outside of the head in the position it reaches during larval life just before it enters the buccal chamber Here it is in the surface territory innervated by the septal nerve

Stage B represents the relation of the parts in the adult Amphioxus The hypophysial organ is housed in the buccal cavity

Stage C is a pre-Bdellostoma condition antedating the formation of a nasobuccal partition The entire head territory of the Amphioxus stage is now housed in the buccal chamber and projects into it along the sagittal plane, forming a partial septum which partly separates the nasal portion of the common nasobuccal chamber into right and left nasal chambers

Stage D represents the Bdellostoma condition The primitive apical territory is now enclosed in a 'nasal capsule,' open below, which is divided into right and left halves by the terminal organ, which forms a complete septum for structures of the capsule The nasal chamber is further separated from the buccal cavity by a horizontal partition The nasohypophysial canal is an extension forward and backward of the nasal chamber

The cranial nerves in man are not, therefore twelve in number, but fourteen In the order of their connection with the brain we may tabulate them as follows

Nerve 1, terminalis	Nerve 8, abducens
Nerve 2, opticus	Nerve 9, facialis
Nerve 3, olfactorius	Nerve 10, acusticus
Nerve 4, septalis	Nerve 11 glossopharyngeus
Nerve 5, oculomotorius	Nerve 12, vagus
Nerve 6, trochlearis	Nerve 13, accessorius
Nerve 7 trigeminus	Nerve 14 hypoglossus

6 COMMENTS ON FUNCTION

We know almost nothing of the function of the terminal organ, of Jacobson's organ, of the hypophysis We are perhaps warranted in assuming that the terminal, olfactory, and septal nerves have to do with special chemical senses, of which above Amphioxus the olfactory sense plays the predominant rôle Next in importance stands the terminal sense organ, which we find from the fishes on, as the organ of Jacobson, a paired sense organ of the nasal chamber which appears reduced in importance as compared with the olfactory organ, although in the Ophidia the reverse appears to be the case The functions of the nerves in the nasal chamber may be divided as follows

Chemical sense	Olfactory nerve	Testing alimentary foods
	Terminal nerve	Testing respiratory foods
	Septal nerve	
Tactile sense	Trigeminal nerve	Testing for solid bodies in the respiratory currents and sensing the pressure and the current flow

Although the chemical sense organs have been housed in the nasal chamber, they have, so far as the structure of the sensory surfaces are concerned, remained in a primitive state These sense organs have not developed accessory parts in such degree as have the eye and ear In the case of the eye, the vitreous body, lens, cornea, lids, etc , have been added to increase the precision or enlarge the range of its functions, there has been added to its light-perceiving function the optical reactions In the case of the ear, there has been a parallel evolution of the primitive function of wave-motion perception by the addition of tone perception, with the cochlea as its anatomical expression In both cases there has been a progressive increase in the number of protective structures as well as of parts serving the increase of precision and enlarged range of function In the case of the chemical sense organs of the nasal chamber there has not been such considerable increase of subsidiary parts or perfection of the sensory structures, and they therefore remain organs whose stim-

uli belong more to the subconscious domain of reaction to the environment than is the case with either the eye or ear Even when conscious attention is directed to the reactions of the nasal organs, they can only partly be brought into the realm of definiteness This is proved by the fact that from the days of Aristotle down to the discovery of the terminal nerve there was never a hint of anything more than an olfactive function Even in man to-day the olfactive function is a vague and uncertain sense in itself and needs, in order to make certain the interpretation of the stimulus, the assistance of other sense organs, e g, the eye or the ear To illustrate, most persons are sure they can recognize the odor of the rose, specifically, a given variety of rose, with whose characteristics they are familiar, but blind folded and lacking tactile stimuli they cannot identify with certainty the source of the odor, often indeed it may call up the memory of violet odor or some other odor It is different with the eye By sight alone and within a considerable range of distances we can recognize any object of definite form which we have seen before The ear stands between the nose and eye with regard to definiteness and certainty of the results of the stimuli While we can 'smell' an odor and not be able to identify it, and can hear a tone and not be able to place it in the scale, we can always recognize objects by sight, by either their form, color, size, motion, or all combined All three senses are quite equally and similarly limited by the upper and lower limits of intensity of stimuli Although the nasal senses lack conscious definiteness, when compared with the eye or ear, they are not on that account less determinative of physiological (and psychological) reactions They are primitive and fundamental senses When stimulated, the nerve reactions, even though subconscious, may be propagated far and wide throughout the nervous apparatus, much like 'sympathetic' reactions Rarely is there an instantaneous response such as so frequently results from stimuli of the auditory nerve The cranial nerves of the nasal chamber have not the intimate associations with the 'voluntary' muscular apparatus that the auditory apparatus has Our knowledge of both the peripheral and central rela-

tions of the four cranial nerves having endings in the nasal chamber is very imperfect Even more fragmentary and obscure is our knowledge of their functions

Winding Way and Valley Road
Cincinnati January 20 1919

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Resumido por el autor, Leslie B Arey

Un mecanismo retinal para la visión eficiente

Las células visuales y el pigmento retinal de muchos vertebrados inferiores exhiben sorprendentes movimientos a la luz y en la oscuridad Los experimentos comunicados previamente han establecido que la rapidez de estos cambios ha sido muy exagerada La validez de la suposición, también muy extendida, respecto a su umbral de sensibilidad extremadamente bajo, ha sido investigada después por el autor Tal determinación es importante en vista de otra suposición discordante que considera que los cambios en la posición de las células visuales a la luz intensa y a la difusa favorecen la visión de los conos y bastones, respectivamente, mientras que los movimientos correspondientes del pigmento retinal también aumentan mecánicamente la eficiencia visual En otras palabras, si los beneficios reputados como adaptativos se derivan de tales cambios fotomecánicos, las reacciones a la luz difusa deben ser esencialmente idénticas con las que se sabe ocurren en la oscuridad total Esta suposición sin embargo, es en su mayor parte gratuita Las reacciones de estos elementos bajo la acción de la luz de intensidad graduada prueban que el umbral de estimulación es notablemente elevado En general, el máximo de reacción hacia la luz aparece primero en una intensidad luminosa que permite justamente la lectura de los caracteres de imprenta ordinarios De aquí que la supuesta sensibilidad fótica elevada de las células visuales y pigmento retinal no queda probada, mientras que las condiciones mecánicas para una visión de la penumbra, teóricamente mas eficiente, se establecen sobre una base experimental

Translation by José F Nonidez
Columbia University

A RETINAL MECHANISM OF EFFICIENT VISION[1]

LESLIE B AREY

The Anatomical Laboratory of the Northwestern University Medical School

TWO TEXT FIGURES

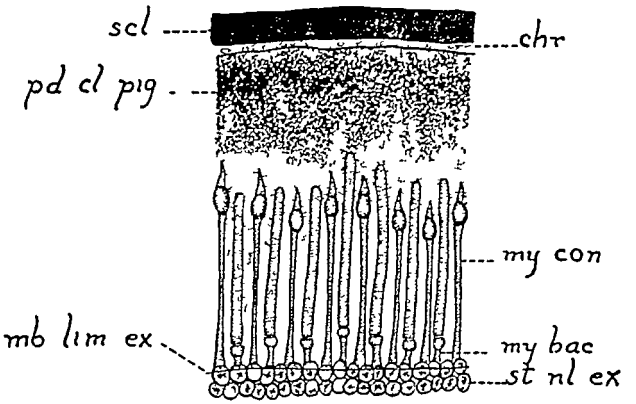
PRELIMINARY

It is a well-established fact of retinal physiology that the visual cells and retinal pigment in many of the lower vertebrates exhibit striking movements in light and in darkness ('15 a, '16), although in man and other mammals such changes apparently are slight ('15 b)

The pigment cells have processes, probably fixed which interdigitate with the visual elements and in which the pigment granules migrate to and fro (figs 1 and 2) The visual cells likewise modify their positions due to the contractility of the so-called myoid, which is that portion of the inner member of the rod or cone between the ellipsoid and the external limiting membrane The extensibility of the myoid is variously developed, but in some animals the extremes of change may be as one is to ten ('16)

There is represented in figures 1 and 2 the condition of the retina, with respect to these changes, as it is characteristically found in a fish, the common horned pout, Ameiurus In darkness (fig 1) the pigment withdraws toward the choroid, thus exposing the visual rods and cones, of the two types of visual elements the cone is extended, whereas the rod is so retracted that its ellipsoid lies close to the external limiting membrane In light (fig 2) the appearance is reversed, the pigment now

[1] Contribution No 69 March 3, 1919 The experimental data were obtained at the Fairport Biological Station while a guest of the United States Bureau of Fisheries



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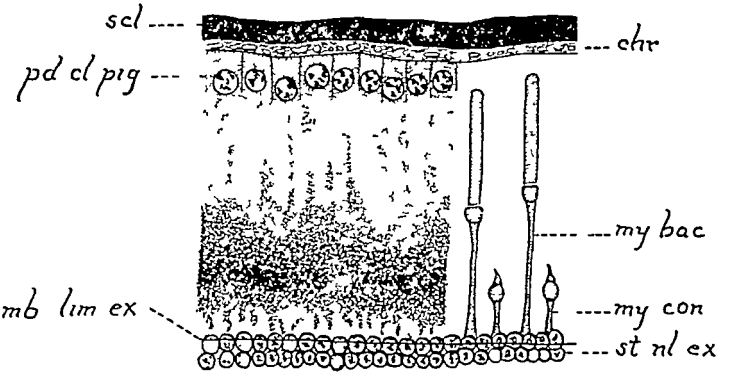

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Fig 1 The effect of total darkness on the position of the retinal pigment and of the visual rods and cones of the fish Ameiurus nebulosus The pigment is withdrawn toward the chorioid, the cone myoid elongates, the rod myoid shortens

Fig 2 The effect of bright, diffuse daylight on the same retinal elements of Ameiurus The pigment moves forward toward the external limiting membrane, thereby masking the visual rods and cones At the right of the figure the positions of these visual cells are indicated—rods elongated, cones shortened

ABBREVIATIONS

chr, chorioid, *mb lim ex*, external limiting membrane, *my bac*, rod myoid, *my con*, cone myoid, *pd cl pig*, base of pigment cell, *scl*, sclera, *st nl ex*, external nuclear layer, *st pig*, pigment layer

pushes outward to mask the visual cells, while these latter occupy mutually reciprocal positions—rods elongated, cones shortened

Such positional changes have been interpreted as of use in furthering efficient vision It seems logical that the masking pigment of the light phase would serve to protect the delicate visual elements from the overstrong influence of light (Chiarini, '06), while the insinuation of such pigmented processes between the individual visual elements effects for the latter a certain degree of optical isolation by acting as an absorbent of dispersed rays refracted from neighboring cells (Garten, '07) From the standpoint of sensory reception, the sharpness of the retinal image is in this way enhanced The withdrawal of the pigment in dim light might be thought to involve a response which allows the visual cells to utilize all the weak light available

Furthermore, there is good reason to believe that the cones are concerned with bright-light vision, the rods with dim light or twilight vision Hence a shortening of the cone in bright light drawing it down nearer the source of illumination while the rod at the same time elongates and is thus moved out of the way, would appear to be a useful maneuver (Herzog, '05, Exner and Januschke, '06) The converse procedure in dim light—by which the rods are shortened and the highly refractile cones, with their lens like ellipsoids no longer masked by pigment, are lengthened—would be equally advantageous (Garten, 07, Arey, '15 a)

For the detailed applications of these apparently adaptive responses the reader is referred to Garten ('07), who gives an extended consideration of the correlations within the vertebrate classes between the morphology, optical qualities, and distributional ratios of the rods and cones on the one hand, and, on the other, their movements together with the migrations of the retinal pigment

Interesting and logical as these speculations may be, they nevertheless lack a sound experimental basis It is certain that the movements as summarized in a preceding paragraph occur respectively in daylight and in darkness but since responses in total darkness cannot be useful in the manner suggested, it is obvious that in order to derive the reputed adaptive benefits

from such photomechanical changes, the responses in dim light must be identical with those demonstrably occurring in the dark This assumption, however, is largely gratuitous

The supposition of an identity between the set of responses ensuing in dim light and in darkness is further complicated by certain conflicting statements and beliefs There is a general impression current that the retinal pigment and visual cells react to the slightest traces of light, this is reflected in the statements of many workers who have feared their results would be impaired unless the strictest precautions were observed

On the basis of actual experimentation, however, the earliest observations are encountered in the writings of Angelucci ('90), who reported that five minutes of candle light caused the frog's cones to be highly shortened, whereas the pigment remained as in darkness On another page, nevertheless, he records that after twenty minutes of twilight the pigment assumed the light position, but the cones are influenced to a less degree[1]

Somewhat later, Pergens ('97) wrote that after a five hours' exposure to colored lights (red, yellow, green, and blue) of an intensity such that colors could be distinguished by an observer after one minute of dark adaptation, the cones of the white fish, Leuciscus rutilis, are strongly retracted The weakest response was reported from the blue—a result, however, not in agreement with Herzog's ('05) later findings on the frog The latter worker found the blue-violet most effective, although it should be added that the duration of exposure employed by him was only two minutes

In a further communication ('99) Pergens confirmed his earlier conclusion that the pigment migrates least extensively in red light (equal intensities being used), but mod fied his previous belief regarding the inefficiency of the blue to provoke cone retraction

Exner and Januschke ('05) performed some experiments, which, unfortunately, are not trustworthy as evidence Specimens of the fish Abramis brama were exposed during the late afternoon, the experiment continuing through the period of failing light and terminating at dusk Examination showed the cones to be in the position characteristic of light

In a few experiments Garten and Weiss ('07) found that light in which colors could not be recognized, acting for five or more hours, caused the cones of the frog to assume a position intermediate between that characteristic of bright light and of total darkness A limited pigment migration was reported as well

These same workers made observations on the fish Abramis brama, contained in a dish lighted by reflection from the cover Two grades of illumination were chosen in one colors held within the container could not be distinguished, in the other they were recognizable According to the results given, in the first grade the cones were maximally retracted in nine retinas and partially so in seven In the second grade the cones were found shortened in all the eight retinas used, whereas the pigment exhibited no noteworthy change except in the sector constituting the ventral one-third of the eye

The foregoing statements reveal the following conditions Angelucci's several pronouncements in the same publication, if not actually contradictory, at least serve to befog the issue The use of colored lights by Pergens was unfortunate, moreover, the reliability of certain of his conclusions is questionable Exner and Januschke's experiments were so ill devised as to furnish no crucial evidence The results of Garten and Weiss suggest an extreme sensitivity of the cones to low light intensities, whereas the pigment patently has a higher threshold Finally, there exist no data concerning the responses of the rods to weak light

It is clear that if the visual elements and retinal pigment are as highly sensitive to mere traces of light, as often has been held, they can assume no useful positions in the ordinary dim light of rod vision, while the utility of a response evoked under conditions of virtual darkness will still await an explanation Accordingly, it was with the intent of learning the true conditions that this investigation was undertaken

PROCEDURE

Essential to success in a determination of this kind is the choice of appropriate experimental animals Previous experience with a variety of forms led to the selection of two fishes and the frog The cones of the golden shiner, Abramis crysoleucas, have large, conspicuous ellipsoids and are so highly mobile that the light-adapted myoid can shorten to one-tenth its maximum length in darkness ('16) The cones of the common grass frog, Rana pipiens, are easy to observe, but show a narrower range of movement, the limits of extensibility being as one is to four The rods of the horned pout, Ameiurus nebulosus, not only are of exceptional size in comparison with the usual diminutive elements of fishes, but they also undergo changes in length in the ratio of one to ten ('16), the value of Ameiurus for experimentation of this sort cannot be overestimated There is a further inherent advantage in the animals chosen, inasmuch as their visual cells remain at a uniform level during the characteristic positional changes The retinal pigment of all three animals exhibits extensive movements in darkness it is confined to a narrow zone at the bases of the pigment cells, whereas in the light it migrates nearly to the external limiting membrane

Temperature is an additional factor which must be carefully controlled, although the necessity of this has been recognized only recently ('16) In dark-adapted fishes a temperature near the freezing point brings about a maximal contraction of the cone myoid, such as is characteristically associated with the action of light, while raising the temperature to the limit which is compatible with life proportionately elongates the myoid Light, however, is so much more effective than temperature that the latter factor does not enter as a complication in the light adaptation of cones The relatively slight quantitative effects of temperature acting in darkness on the position of the rods and on the distribution of pigment may also be disregarded In the frog, on the contrary, high and low temperatures evoke a maximal expansion from the pigment during dark adaptation, complete contraction being obtainable only at an intermediate grade of about 15°C , the cones, however, are shortened at the

upper temperature limits alone From these statements it follows that to obtain significant results from experimentation upon frogs a temperature of about 15°C must be maintained, while with fishes ordinary summer temperature of 22°C, or higher, is favorable

Experiments were conducted in a large, windowless room into which weak daylight of a non directive nature could be introduced from a second room, the latter, in turn, received its light directly from windows located at the end far from the single communicating door Animals were tested under five different conditions of illumination 1) total darkness, 2) light in which the presence of objects could just be determined, 3) light of an intensity which allows the certain identification of bright colors, 4) light which just permits the reading of ordinary journal print, 5) bright, diffuse daylight Exposures lasted two or three hours or more

As experience proved, these seemingly rough criteria of light intensity are sufficiently accurate for the purposes required, with a little practice such grades can be kept fairly uniform Permanent preparations of Perenyi-fixed, paraffin infiltrated sections served as a basis for study

To further brevity and clearness, the results obtained from experimentation will be condensed to mere summaries

EXPERIMENTATION

A Retinal pigment

1 Frog In total darkness, and in light of just sufficient strength to allow objects or colors to be discerned, the pigment lies in a narrow stratum near the choroid When the illumination is increased just sufficiently to allow the reading of ordinary print, the pigment, for the most part, becomes expanded, in some cases completely, in others in a zone only three-fourths the maximal breadth

[1] For Ameiurus another intensity—one which enables ordinary print to be easily read—was also utilized

2 Ameiurus The pigment of this fish is at first more sensitive than that of the other animals studied, migration being distinctly initiated in many (or perhaps most) individuals at an intensity by which the presence of objects can be detected At the next grade (colors distinguished), expansion is well advanced, although the pigment does not extend the maximal distance, for it is dense at the cell bases, but sparse distad In light in which one can just read, the expansion is nearly complete, but does not become maximal until the illumination is sufficient to allow easy reading

3 Abramis The effect of light is not apparent until it is of such a strength that colors can be recognized At this intensity the pigment in about half the individuals was essentially in a condition of greatest contraction, the remainder showed the pigment well started, but not extended at most more than half the way to the external limiting membrane Owing to a sudden failure in my supply of animals at the end of the season, I secured but few observations on the effect of light which makes reading possible, the pigment in those animals studied, however, was in a state of partial expansion only, so that it appears safe to conclude that stronger illumination is necessary to allow expansion to proceed to completion

B Visual cells

1 Cones The cones of Abramis and the frog remain fully elongated, in the characteristic dark positions, until the illumination is so increased that colors are recognized At this intensity the cones of Abramis show distinct indications of incipient retraction, while those of the frog are less than half their former length When illuminated sufficiently to allow reading, the frog's cones shorten maximally, the cones of the few Abramis studied[3] were likewise greatly shortened, but not completely so

[3] As on the tests on the retinal pigment of this animal, further experimentation would have been desirable, but the supply of available material suddenly ceased

2 Rods Until an intensity is used at which printed matter can be read, the rods of Ameiurus remain in the typical shortened condition of darkness, at about this grade, however they apparently begin to elongate slightly Any considerable degree of lengthening must first appear only in stronger light

DISCUSSION AND CONCLUSION

These results, as compared with the findings of Garten and Weiss (compare p 347), indicate that there exists a rather lower degree of photic sensitivity in the visual cells and retinal pigment than they maintain Nevertheless, it must be apparent to all, not only that in the subjective choice of arbitrary grades of light a variable personal factor enters, but also that the description of stages thus chosen cannot be expressed in accurate terms It is possible, of course, for any one person to standardize these grades fairly well for his own experiments, on the other hand, the determination of a critical intensity, such as that in which 'colors can be distinguished,' depends on one s individual acuity in color discrimination, on the brightness of the test colors, and on the decision as to whether these colors are to be just distinguishable with intense scrutiny or to be identified with ease In any event, it appears that my first grade of illumination (that in which objects were discernible) was undoubtedly of lower intensity than the weakest employed by Garten and Weiss (compare p 347), it lay far below the point where colors cease to be recognizable, this latter constituting their lowest grade

Moreover, it is not impossible that the intensity of light to which their animals were actually subjected was higher than Garten and Weiss supposed Their animals contained in a 'spacious basin,' received light (electric) reflected downward from the cover After the experimenter had accustomed his eye to total darkness for five minutes, the condition of illumination was judged by looking downward into the dish at colors placed directly over the surface of the water As to whether this is a method which tends toward underestimating the light

conditions actually obtaining in the basin, the reader may decide for himself

There is one further circumstance which on casual consideration might be held responsible for the quantitative divergence between my results and those of Garten and Weiss They continued their experiments in most cases for five hours, while my determinations lasted on the average perhaps three hours It seems plausible that the long-continued action of very weak light might register an effect not manifest in shorter periods That this possibility is not operative in the cases under consideration follows from certain other observations of Garten and Weiss They report maximal cone retraction in the fish after the weakest grade of light employed had acted in one series for three hours and in another crucial series for one and one-half hours Garten also records that in light too weak to distinguish colors by, a shortening of the cones occurred ('eintritt') in one hour

The facts developed in this investigation may for convenience be consolidated into the following statement although responses of the visual cells and retinal pigment may be initiated at lower intensities of light, an approach to a maximal response is first elicited at an intensity which permits the reading of ordinary print *This signifies that the threshold of stimulation of the visual cells and retinal pigment is high, or, in other words, the assumed great photic sensitivity of these elements is disproved* Furthermore, since the responses in weak light are substantially identical with those in darkness, the mechanical conditions are present for a theoretically more efficient dim-light and bright-light vision, as postulated (compare p 345)

SUMMARY

The threshold of stimulation of the visual rods and cones and of the retinal pigment, at which they exhibit their characteristic photomechanical changes, is high

The alleged great sensitivity of these elements to light of extremely low intensity is consequently disproved

Although responses may be initiated at lower intensities in general an approach to a maximal light response is first elicited at an intensity which makes ordinary print legible

Since the responses in dim light approximate those in total darkness the mechanical conditions are present for a theoretically more efficient dim light and bright-light vision than would otherwise obtain This increased efficiency depends upon the assumption by these elements of correlative advantageous positions

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Contribución al estudio de los reflejos vasomotores

La estimulación de los nervios sensoriales en perros anestesiados con éter o cloroformo produce generalmente un aumento en los movimientos respiratorios, cuando la narcosis no es muy fuerte o cuando no se emplea el curare Este aumento de movimientos respiratorios produce una disminución de la presión sanguinea y cuando son pronunciados no se puede obtener ningún reflejo presor, aun estimulando fuertemente Con narcosis fuerte o compresión del cerebro no se produce la disminución de la presión sanguinea debida a esta causa La interferencia mecánica con la circulación es la causa de esta disminución, puesto que se elimina abriendo el tórax Este fenómeno complica seriamente los experimentos sobre los reflejos vasomotores En los perros anestesiados con éter o cloroformo o con el cerebro comprimido, una débil estimulación de los nervios produce generalmente una disminución, y una fuerte estimulación un aumento en la presión sanguinea La frecuencia de la estimulación ejerce efectos sobre el reflejo, cuando es rápida se obtiene un aumento, y con menor rapidez una disminución Los nervios más voluminosos responden de un modo más marcado al estímulo que los menores La estimulación de la piel, músculos e intestino origina generalmente una disminución en la presión sanguinea, pero si se estimula la piel de un modo violento y extenso se produce un aumento en dicha presión Bajo la acción de la morfina y el curare, por el contrario, un aumento en la presión tiene lugar generalmente, aunque con la morfina la estimulación débil puede producir una disminución de la misma La influencia de las glándulas endocrinas sobre los reflejos vasomotores no es clara (veáse sin embargo Pearlman and Vincent "Endocrinology," en prensa) El cambio de reflejo en la estimulación de los nervios somáticos se produce principalmente por los efectos sobre los vasos sanguíneos del área esplácnica

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A CONTRIBUTION TO THE STUDY OF VASOMOTOR REFLEXES

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NINETEEN FIGURES

CONTENTS

1 INTRODUCTION

General blood pressure is affected reflexly by central stimulation of various sensory nerves (reflex vasomotor action) This subject has been studied already by a number of authors A complete list of the older investigations may be found in Tigerstedt's Lehrbuch der Physiologie des Kreislaufs,[41] papers by Asher[1] and Bayliss[2] in Ergebnisse der Physiologie and in Nagel's Handbuch der Physiologie (Hofmann[11]) The history up to November, 1914, is given by Vincent and Cameron[43] As to more recent important investigators of this problem, we may refer to Porter,[29–31] Martin[6] Ranson,[35–36] Gruber,[8 9] and their respective co workers, also to Domitrenko[5] and Hunt[15 16]

Even among these recent investigators there seems to be considerable difference of opinion as to what may be regarded as the usual or normal response to afferent impulses Thus Porter

and Quinby[34] say "It is sometimes urged that in shock the blood-pressure falls instead of rising on stimulation of afferent nerves This abnormal reaction was observed in several of our experiments" This statement clearly involves the assumption that a rise is the normal effect, though it is recognized that the fall is a not very unusual occurrence Vincent and Cameron[43] seem to be of opinion that the usual effect of stimulating the central end of the cut sciatic nerve is a rise, and the fall due to a pure vasomotor reflex is rather rare So also Hunt On the other hand, Martin and Lacey[23] having observed regularly a definite drop in blood-pressure by weak stimulation and a rise by far more strong stimulation, became doubtful of the truth of the generally accepted doctrine that pressor responses are the normal results of sensory stimulation

These differences of opinion must be due to some factor or factors other than the strength of stimulus Besides the factors most usually considered, such as different modes of stimulation, different nerves, different conditions of the same nerve, different narcotics, drugs, etc , there are two important considerations recently brought forward which unmistakably affect the vasomotor reflexes or complicate the problem of their elucidation

In 1915 Vincent and Cameron[43] called attention for the first time to a fall of blood-pressure caused by increased respiratory movements They write "While anaesthesia is fairly complete the effect of stimulating the central end of the cut sciatic nerve is a pure and distinct rise As the effect of the anaesthetic begins to pass off, the effect of stimulation will be a rise of blood-pressure followed by a more or less pronounced fall Respiratory movements will now be found to have been markedly increased, and the extent of the fall of pressure appears to be at any rate proportional to the violence of the respiratory activity"

Martin and Lacey[23] investigated the influence of the interruption of the primary current at widely varying rates, but failed to notice any effect, as also did Hunt[14] in his earlier work Only quite recently it was clearly pointed out by Gruber[8] that with the same strength of stimulus, pressor and depressor results

were obtainable by varying the rate of stimulation from 1 to 20 stimuli per second This was later incidentally confirmed by Hunt [16]

Thus, for the investigation of the complicated problem of vasomotor reflexes, it became very necessary to investigate each possible factor separately In this way only would one be able to answer correctly for the normal vasomotor response

The present investigation was undertaken for the purpose of studying some of the factors separately, of confirming previous investigations, and of trying, if possible, to reconcile contradictory views as to the conditions which determine any particular vasomotor response

We beg to acknowledge our indebtedness to Mr John Carmichael for his valuable assistance in all our experiments

2 THE INFLUENCE OF RESPIRATORY MOVEMENTS UPON BLOOD PRESSURES

It is well known that the respiratory center can easily be affected by central stimulation of sensory nerves Thus Howell [13] writes in his Textbook of Physiology that 'stimulation of any of the sensory nerves of the body may affect the rate or the amplitude of the respiratory movements' But no mention is made of the influence of these movements upon blood pressure The same applies to other text-books except Starling's,[25] in which we find, "The increased respiratory movements will also aid the venous circulation and have a similar effect in increasing the systolic output," which would necessarily bring about a *rise* of blood pressure But, 'A constant and immediate result of exaggerated respiratory movements is a fall of blood pressure," and not a rise as Vincent and Cameron pointed out They found that "the extent of the fall of pressure appeared to be at any rate largely proportional to the violence of the respiratory activity," that the fall of blood pressure was 'brought about by performing rapid artificial respiration by compression of the thorax," that deep voluntary breathing in the case of the human subject produced a regular and pronounced lowering of the blood pressure," that the more widely the thorax is opened

the more the fall of pressure tended to become replaced by a rise," and that the effect of artificial respiration was "a rise, and not a fall, when the animal was under curare," i e , when a stop was put to the spontaneous respiratory movements Thus the fall of blood-pressure as a result of increased respiratory movements seems to have been sufficiently established

By the majority of previous observers curare was thought to be an indispensable drug in the study of the problem of vasomotor reflexes, with or without any consideration of its action on the vasomotor center itself But we know that narcotics and other drugs are not always free from influence upon these reflexes, as pointed out by various previous investigators,[14] [29] [3] and, therefore, in experimental work they should be reduced to as few as possible or altogether eliminated (Vincent and Cameron) The change in character of the respiratory movements, especially their increase, becomes thus an almost unavoidable complication in the study of vasomotor reflexes when curare is not used and the narcosis is not deep enough If this complication be left out of consideration, erroneous conclusions may be reached

Since the appearance of Vincent and Cameron's paper several writers have referred to the influence of the increased respiratory movements Unfortunately, they are not in complete harmony with one another Ranson and Billingsley[35] [36] say, "With stronger stimulation the greatly increased respiratory movements may no doubt play an important part in the drops in blood-pressure," but Gruber and Kretschmer[9] write that their "experiments do not support Vincent and Cameron's theory that the fall in blood-pressure is brought about by movements of respiration which interfere with the heart's activity" This latter statement seems to deny definitely the respiratory rôle upon blood-pressure Vincent and Cameron did not positively deny that there is a true vasomotor fall of blood-pressure under certain conditions as a result of central stimulation of afferent fibers But they insisted, and rightly, too, that many apparent vasomotor falls are really due to increased respiratory movements We shall see later that the fall of blood-pressure with weak stimuli is a commoner occurrence than Vincent and

Cameron were inclined to believe At any rate, the matter is so important that we have repeated the experiments to test the effects of respiration on blood pressure

We have stimulated electrically the central stump of several cut nerve trunks (saphenous, tibial, peroneal, sciatic, ulnar, and median) with various strengths of stimulus

When the narcosis (with ether or chloroform) was not deep enough, the respiratory movements were always increased by strong stimulation The most frequent response of the blood-pressure to stimulation, e g, of the sciatic nerve, may be illustrated by figure 1

With weak stimulation there is practically no increase of respiratory movements either in amplitude or in frequency, and the blood-pressure is either a fall or a fall followed by a more or less marked rise When stronger stimuli are applied, the respiratory movements increase either in amplitude or in frequency, or in both, and the blood-pressure rises instead of falling, and is followed by a marked fall The rise of blood pressure increases usually in proportion to the development of the strength of stimulation

In figure 2 the anesthesia was made much deeper with the same animal as in figure 1, and stimuli of several strengths were applied to the same nerve

There is very little increase of respiratory movements on each stimulation, and the response of blood pressure is also small in degree The latter, as seen from the figure, is either a fall or a rise according to the strength of stimulation, and the marked fall after the rise which is observed in figure 1 simultaneously with the increased respiration cannot be seen This suggests at once that the marked fall accompanied by remarkably increased respiratory movements might be ascribed, at any rate mainly, to the influence of the latter movements caused by sensory stimulation Moreover, under brain compression it is not very difficult to stop the respiratory movements entirely, and in this case only very strong stimulation will initiate spontaneous respiratory movements Under these conditions a fall of blood pressure of the same character as that observed with an in-

creased respiration never occurs Thus it would not be unreasonable to assume that figure 2 shows real vasomotor reflexes, even though weak, not complicated by the increased respiratory movements, while figure 1 represents the vasomotor reflex masked by the effects of increased respiration

It is often very difficult or almost impossible to obtain any rise of blood-pressure when the respiratory movements are very violent In those cases a marked fall is the only result of central stimulation of afferent fibers

How these increased respiratory movements affect the blood-pressure was very carefully investigated by Vincent and Cameron After pointing out several possible causes, they came to the conclusion that this fall is due to direct mechanical interference with the heart's action and with the return of the blood to the heart

In order to confirm this theory, we opened the thorax in the middle line as did Vincent and Cameron, and found that the falls disappeared The contrast is clearly shown in figures 3 and 4

In these two cases the same nerve of the same animal was stimulated with the same strength of stimulus, in figure 3 in the intact animal and in figure 4 with thorax open

In addition to opening the thorax we cut both vagi, both phrenici, and as many intercostals as possible on both sides, without obtaining very different results from those obtained by merely opening the thorax

In a very few cases a marked fall of a similar character to that due to the increased respiratory movements, was observed in animals with thorax wide open It was, however, soon discovered that this fall was produced by compression of the inferior vena cava by the heart which became more freely movable than before through opening the thorax The heart fell back upon the soft-walled vein, and thus diminished the flow of blood to the right heart

But it is certainly true that by means of almost pure vasomotor reflex, i e , without any or with very little increase of respiratory movements one can obtain a marked fall preceded by a rise, as shown in figure 5

Therefore we do not conclude that such a fall of blood pressure is always produced by increased respiratory movements But the important point for us at present is the undoubted fact that increased respiratory movements can and do cause a fall of blood pressure, and that this fall can be easily eliminated by opening the thorax sufficiently wide

These observations, along with various others quoted above from the paper by Vincent and Cameron both on animals and on human subjects, confirm fully their statement as to the occurrence of a fall of blood-pressure brought about by increased respiratory movements, and probably explain the nature of this fall We believe that the increased respiratory movements caused by sensory stimulation form a very important complication which has often led to misunderstanding of the true vasomotor reflexes

Gruber and Kretschmer, as mentioned before, deny this respiratory effect upon blood pressure They used a slow rate of stimulation and the fall of blood-pressure was the usual effect But the fall is generally thought to be a result of weaker stimulation, and they do not deny that the increased respiratory movements to a certain degree cause a fall of blood pressure when the stimulus is strong enough as to produce them

Our experiments were made on thirty-three dogs

3 THE EFFECT OF THE STRENGTH OF THE STIMULUS UPON VASOMOTOR REFLEXES

After repeated experiments by numerous investigators the generally accepted view as to the effect upon the vasomotor reflexes of different strengths of stimulus seems to coincide with Knoll's[19] original statement, i e , that a depressor effect is usually the result of a weak stimulation while a pressor effect follows, as a rule a stronger stimulation Reid Hunt[14] pointed out that weak stimulation was one of the methods of obtaining a reflex fall of blood pressure, and Vincent and Cameron noticed the same fact

Among more exhaustive investigations on this point we should refer to those by Porter,[29–31] Martin,[32–36 40] and their respective

co-workers The former writer seems to regard a rise of blood-pressure as the normal vasomotor response, while the latter holds a different view Martin and Lacey's[23] experiments were conducted on cats either under brain pithing, decerebration or brain compression, or under ether or urethane The nerves stimulated were the sciatic, radial, median, ulnar, and saphenous The results of their experiments were very definite "In every one of the experiments the stimulation was repeated many times over a range of stimuli from the threshold value to three or four times the threshold Well-marked drops of pressure followed all such stimulations," save in one exceptional case Thresholds for pressor reflexes were much higher than those for depressor reflexes Thus the experiments of these workers support Knoll's statement

Our own experiments consisted in stimulating various nerves (sciatic, tibial, peroneal, saphenous, median, ulnar, and vagus) with induction shocks on dogs under ether, chloroform, and brain compression As to the method, we have to mention that the different effects of weak and strong currents, respectively, were satisfactorily attained by means of sliding the secondary coil up to or away from the primary, but that on many occasions more than one battery was used to obtain a stronger stimulus The rate of stimulation was 38 to 54 in a second

The fall of blood-pressure due to the increased respiratory movements being taken into consideration, the main results of our experiments may be summarized as in the following table

ANAESTHETICS	FALL WITH WEAK STIMULATION RISE WITH STRONG STIMULATION	FALL WITH WEAK AND STRONG STIMULATION	RISE WAS ONLY RESULT OF STIMULATION
Ether	20	2	4
Chloroform	14	4	4
Brain compression	12	2	0
Total	46	8	8

The term 'fall' in the table comprises also a fall followed by a rise and 'rise' also a rise followed by a fall

In forty-six cases out of sixty-two in total weak stimulation produced a fall or a fall followed by a rise, and strong stimulation caused a rise or a rise followed by a fall A typical response is shown in figure 6

The animal was under ether and the thorax was very wide open in the middle line in order to eliminate the disturbance from increased respiratory movements Figure 7 shows a similar response under chloroform

In the remaining sixteen cases the response was either a fall or a rise through all strengths of stimulation which we used, and the different effects with weak and strong stimulation were not observable

Thus it does not seem to us unreasonable to conclude that weak stimulation of the central stump of the cut nerve produces usually a fall of blood-pressure and a strong stimulation produces usually a rise

From these conclusions it may naturally be understood that from the threshold of stimulation up to a certain point the fall of blood pressure increases with the development of the strength of stimulus, and then the fall gradually decreases until a neutral point is reached, where the vasoconstriction and dilatation just counterbalance each other, and finally the rise appears, which increases usually with the increase of the strength of stimulus, but cannot continue very long, since powerful stimuli would elicit vigorous reflex movements of the animal and obscure the true vasomotor reactions unless indeed the animals were deeply under curare As we have been unable so far to find any attempt by previous investigators except Stiles and Martin[40] to describe this rather peculiar course of vasomotor responses, we though it worth while to emphasize it in this place (fig 8)

In our experiments we have employed also stimuli of other kinds than electric induction shocks, namely, mechanical, thermal, and chemical In this series thirty-eight out of sixty-seven stimulations were effective, and of these thirty-five caused a fall of blood-pressure and only three produced a rise As the calibration of these stimuli was not so practicable as with induction shocks, we cannot draw any very positive conclusions, but we

are inclined to believe that a greater number of pressor responses could be obtained if we could improve the method of stimulation so that the sensory fibers might be stimulated more strongly

It thus appears from our experiments that the depressor effect of weak stimuli is much more common than Vincent and Cameron thought, though these observers were careful not to deny its occurrence Reid Hunt,[16] in a recent paper, seems to have had the same difficulty that Vincent and Cameron encountered in obtaining the depressor effect of weak stimulation, which he ascribed to the different frequency of stimulation they employed

The fact that a weak stimulation of a sensory nerve causes, as a rule, a reflex fall of blood-pressure and a strong stimulation a reflex rise, together with the statement of Bayliss[3] that the orthodox effect due to the stimulation of the depressor nerve (nerve of Cyon[5]) can be converted into a rise by the action of strychnine, led us to inquire whether a pressor response could be obtained by strong stimulation of the depressor nerve So far as our results inform us, neither such a strong current as would injure the nerve nor induction shocks up to eighty per second frequency could reverse the depressor response The response to the stimulation after injection of strychnine was sometimes increased and sometimes decreased, but the reversal of the response did not appear in our experiments even with a dose which caused general convulsions on weak stimulation

4 THE INFLUENCE OF THE FREQUENCY OF STIMULATION UPON VASOMOTOR REFLEXES

That the frequency of stimulation has a certain effect upon vasomotor reactions seems to have been known to the older investigators In 1883 Kronecker and Nicolaides[20] noticed that the vasomotor centers could more easily be affected by changing the frequency of stimulation than by changing its strength They write "One can never attain such a strong vasoconstriction by increasing the intensity of the stimulating current as by increasing the frequency of the current of moderate intensity" We have not been able to consult the original paper of these writers

From a reference in Ergebnisse der Physiologie by Asher,[1] it is not clear whether the stimulation was directly upon the nerve centers or reflexly through afferent nerves

But the credit of pointing out clearly that the frequency of stimulation has an effect upon vasomotor reflexes must be ascribed to Gruber.[8] This writer remarks "That summation takes place with rapid rates of stimulation is undisputable, but it does not seem probable where the strength is more than 400 times threshold that the phenomenon of summation can explain the different effect obtained with these rates of 1 per two seconds and 20 per second interruptions." The similar effect of frequency of stimulation was afterward proved incidentally by Reid Hunt,[10] who considers it convenient to use the infrequent rate of stimulus to obtain a reflex fall of blood pressure

Our experiments on this subject have been carried out on dogs with rates of stimuli of 1, 2, 5, 10, 20, 40, and 80 per second upon various nerves, under chloroform and curare or under brain compression. Though our results were not so conclusive as those obtained by Gruber (in fifteen out of forty stimulations similar results to his were obtained) still we do not hesitate to ascribe an important rôle to the frequency of stimulation. According to Martin's[2] investigations the intensity of stimulation in Z-units is directly proportional to that of the current in the primary circuit. We arranged the apparatus in such a way as to get a current of a certain strength and one ten times stronger as we desired. With the former current we obtained a fall by stimulating five times per second, and a distinct rise by stimulating ten times per second, while with the latter current we observed a fall with the rate of stimulus one per second and a rise with five per second stimulations. A selected record is shown in figure 9, where one and the same nerve was stimulated with the same intensity but with different frequency.

Much more remarkable were the rates of stimulation at which the maximum pressor response was reached

	RATE OF STIMULI PER SECOND						
	1	2	5	10	20	40	80
Number of experiments whose maximum pressor response was reached at the rate of stimuli mentioned above	0	0	0	4	18	12	4

As is seen from the table, in 78 9 per cent the maximum response is reached between twenty to forty per second stimulation, and in one-third at the rate of forty per second Beyond these points the effect increased only in four cases This phenomenon may be seen also in figure 9

Kronecker and Nicolaides[20] observed the fact that the effect of stimulation of the vasomotor centers increased with the frequency of stimuli up to twenty to thirty per second, but not beyond this point Tur[42] also pointed out that the effect of stimulation of the lingual nerve increased until the stimuli reached forty per second, beyond which, however, the effect diminished These observations coincide fairly well with our own

5 EFFECTS UPON VASOMOTOR REFLEXES OF STIMULATING NERVE TRUNKS OF DIFFERENT CATEGORIES (SENSORY, MOTOR, AND MIXED NERVES) AND OF DIFFERENT SIZES

According to the investigations of some authors, different nerves, apart altogether from the depressor nerve, respond differently to central stimulation Hofmann,[11] in Nagel's Handbuch, says "There are single nerves, which for the most part (glossopharyngeal) are depressor, and others which are exclusively (splanchnic) or preponderatingly (sciatic, facial, infraorbital, cervical nerves) pressor" Vincent and Cameron studied the effect of stimulating the main trunk of the sciatic, as well as its common peroneal, lateral cutaneous, and purely muscular branches, the saphenous, median of axilla, the hypoglossal, the glossopharyngeal, the superior laryngeal, and the vagus But the different nerves all produced similar or comparable results on the blood-pressure They were strongly tempted to the hypothesis that an equivalent stimulation of a roughly equal number of afferent fibers will yield similar reflexes

Our experiments also have led to the conclusion that there is no essential qualitative difference between the various nerves subjected to stimulation (sciatic, tibial, peroneal, median, ulnar) A possible exception may be made in the case of the saphenous It may be that there is a greater tendency to a fall on stimulating this nerve than in the case of others Whatever the nerve may be, whether a purely sensory nerve, as the saphenous, a mixed nerve, as the sciatic, peroneal, tibial, ulnar, or median, or a purely motor nerve, such as a muscular branch of the femoral, the course of response to weak and strong stimulation was in most cases a fall and a rise as already described in section 3

A few examples are quoted in tabular form where a selected purely sensory nerve and a purely motor or a mixed nerve apparently of the same size were stimulated in turn under entirely similar conditions

Sensory and motor nerves compared

ARRANGEMENTS OF STIMULATION	RIGHT SAPHENOUS	BRANCH OF RIGHT FEMORALIS
1 battery coil at 30 cm 15 seconds	−4 mm Hg	0 mm Hg
1 battery coil at 25 cm 15 seconds	−10 mm Hg	−1 +2 mm Hg
1 battery coil at 20 cm 15 seconds	−10 +2 mm Hg	−4 +2 mm Hg
1 battery coil at 15 cm 15 seconds	+4 −12 mm Hg	+1 0 mm Hg
1 battery coil at 10 cm 15 seconds	+6 −11 mm Hg	+8 −2 mm Hg
1 battery coil at 5 cm 15 seconds	+16 −18 mm Hg	+14 −12 mm Hg
1 battery coil at 0 cm 15 seconds	+22 −14 mm Hg	+26 −14 mm Hg

(From experiment 47 Under brain compression)

The sign − means a pure fall of blood pressure + a pure rise and − + or + − a mixed response namely a fall followed by a rise or a rise followed by a fall respectively

Sensory and mixed nerves compared

ARRANGEMENTS OF STIMULATION	RIGHT SPHYGMUS	RIGHT PERONEAL
1 battery coil at 30 cm 15 seconds	0 mm Hg	0 mm Hg
1 battery coil at 25 cm 15 seconds	−6 0 mm Hg	−1 0 mm Hg
1 battery coil at 20 cm 15 seconds	+2 −10 mm Hg	−4 +4 mm Hg
1 battery coil at 15 cm 15 seconds	+6 −12 mm Hg	+2 −6 mm Hg
1 battery coil at 10 cm 15 seconds	+8 −12 mm Hg	+14 −10 mm Hg
1 battery coil at 5 cm 1 second	+22 −10 mm Hg	+20 −14 mm Hg
1 battery coil at 0 cm 15 second	+22 −14 mm Hg	+2 −20 mm Hg

(From experiment 47 Under brain compression)

With the increase of the strength of stimulus the respiratory movements also increased, though not very markedly, and therefore some of the falls following the rise on stronger stimulation might have been more or less due to this complication But in the main it seems that the purely sensory nerves have somewhat lower threshold than other kinds of nerves (fig 10)

Whether this is due to a large number of afferent fibers contained in the sensory nerve than in those of the other kinds of the same size could only be decided by more numerous experiments and more elaborate methods than those we have employed, as, for example, the measurement of resistance of each nerve and more satisfactory methods of controlling the intensity of stimulation in each case

In connection with the problem as to different kinds of nerves we have studied the influence of the size of the nerve upon vasomotor reflexes The hypothesis of Vincent and Cameron is quoted at the beginning of this section A similar problem was taken up also by Stiles and Martin,[40] who compared the effect of stimulating two nerve paths at the same time with that of exciting each by itself They found that "stimulation of two afferent paths at the same time has often a more marked vasomotor effect than the stimulation of either path alone with an equivalent strength of current The degree of summation was only moderate" This shows that the stimulation of a larger number of afferent fibers will produce often a more marked effect than that of few fibers

We stimulated two nerves of the same category but of different sizes separately one after another under conditions as similar as possible, a different number of afferent fibers being assumed to be present in the nerves of different sizes

The results may be represented as follows, page 369

These few examples show that the results were not very conclusive We can say only so far with some confidence that when the responses were in the same sense, i e , when the fall or the rise was the result of corresponding equivalent stimulations, the reflex change of blood-pressure was on the whole more marked with the nerve of larger size than with those of smaller size (fig 11)

Mixed nerves compared with each other

ARRANGEMENTS OF STIMULATION	RIGHT SCIATIC	RIGHT PERONEAL
1 battery coil at 30 cm 15 seconds	0 mm Hg	0 mm Hg
1 battery coil at 25 cm 15 seconds	−6, 4 mm Hg	−10 0 mm Hg
1 battery coil at 20 cm 15 seconds	−14 0 mm Hg	−8 4 mm Hg
1 battery coil at 15 cm 15 seconds	12 −22 mm Hg	8 −6 mm Hg
1 battery coil at 10 cm 15 seconds	18 −16 mm Hg	12 −10 mm Hg
	RIGHT SCIATIC	RIGHT TIBIAL
1 battery coil at 25 cm 15 seconds	−2, 2 mm Hg	−2 2 mm Hg
1 battery coil at 30 cm 15 seconds	−6 4 mm Hg	−2 0 mm Hg
1 battery coil at 15 cm 15 seconds	16, −18 mm Hg	10 −8 mm Hg
1 battery coil at 10 cm 15 seconds	22 −22 mm Hg	20 −16 mm Hg

(From experiment 47 Under brain compression)

Sensory nerves compared with each other

ARRANGEMENTS OF STIMULATION	RIGHT SAPHENOUS	A BRANCH OF THE RIGHT SURAL
1 battery coil at 25 cm 10 seconds	0 mm Hg	0 mm Hg
1 battery coil at 20 cm 10 seconds	−6 0 mm Hg	0 mm Hg
1 battery coil at 15 cm 10 seconds	−18 0 mm Hg	−2 0 mm Hg
1 battery coil at 10 cm 10 seconds	−22 0 mm Hg	−8 0 mm Hg
1 battery coil at 5 cm 10 seconds	−18, 0 mm Hg	+2 0 mm Hg
1 battery coil at 0 cm 10 seconds	+4 0 mm Hg	−1 0 mm Hg
	RIGHT SAPHENOUS	A BRANCH OF THE RIGHT SAPHENOUS
1 battery coil at 25 cm 10 seconds	0 mm Hg	0 mm Hg
1 battery coil at 20 cm 10 seconds	−4 0 mm Hg	0 mm Hg
1 battery coil at 15 cm 10 seconds	−8 0 mm Hg	0 mm Hg
1 battery coil at 10 cm 10 seconds	−4 0 mm Hg	−4 0 mm Hg
1 battery coil at 5 cm 10 seconds	+4, 0 mm Hg	−4 0 mm Hg
1 battery coil at 0 cm 10 seconds	+2 0 mm Hg	−6 0 mm Hg

(From experiment 48 Under brain compression)

These conclusions coincide with the experience of Stiles and Martin and lend some support to the hypothesis of Vincent and Cameron

It may not be amiss to add to these conclusions that in few cases when the stimuli were very strong the smaller nerve reacted more vigorously than the larger one which phenomenon may perhaps be explained partly by different resistances of different-sized nerves to currents of similar strength

Thus, so far as our experiments go, we are inclined to conclude provisionally that among nerves of different categories there are no essential qualitative differences of response, and the greater the number of afferent fibers stimulated, the more marked is the response of blood-pressure within a limited range of strength of stimulation

6 VASOMOTOR REFLEXES FROM NERVE TERMINATIONS

Several investigators have stimulated the nerve terminals instead of the nerve trunk itself

When we apply a stimulus to a surface such as the skin we should bear in mind that we may actually be stimulating either the end-organs alone or these structures as well as the nerve fibers, according to the mode of stimulation Any physiologically appropriate stimulus, though mild, applied to the end-organs would give rise to more highly effective impulses than inappropriate ones Thus the study of vasomotor reflexes in response to stimulation of the sense organ with its most appropriate stimulus is highly desirable But even with other kinds of stimulation we may learn much that is valuable, because any stimulus which plays a part in our normal daily life comes usually through the end-organs on the outer or inner surface of the body, and not by way of exposed nerve trunks, as in the foregoing experiments

The skin, the mucous membrane of the nose, muscles, the intestine, and other abdominal organs were employed frequently by previous investigators We have selected the skin, muscles, and the intestine as representative of regions containing different modes of nerve endings The stimuli used were mechanical (incision, scratching, pinching, kneading), thermal (hot or boiling water and cold water or lumps of ice), chemical (10 per cent solution of sulphuric acid), and electrical (induction shocks of various strengths) The animals (dogs) were under ether, chloroform, or brain compression

The results of a first series are presented in the following tables

Mechanical stimulation of the skin

ANESTHESIA	STIMULATED PORTION	MODE OF STIMULATION	REFLEX RESPONSE OF BLOOD-PRESSURE		
			No effect	Fall	Rise
Ether	L inn thigh	Pinching	0	2	0
	R inn thigh	Scratching	2	5	0
	R inn thigh	Incision	0	6	0
Chloroform	L inn thigh	Pinching	1	1	0
	R inn thigh	Scratching	2	3	0
	Abdomen Over saphenous ulnar and sciatic nerves	Incision	3	4	0
Brain compression	L inn thigh	Scratching	0	2	0
	R inn thigh	Incision	0	3	0
Total			8	26	0

Thermal stimulation of the skin

Ether	L inn thigh	65 C—boiling water	0	5	0
	L inn thigh	Ice—15 C water	3	0	0
Chloroform	L inn thigh	Boiling water	2	3	0
	L inn thigh	Ice	0	1	0
Brain compression	L inn thigh	Boiling water	0	1	1
	L inn thigh	Ice	2	0	0
Total			7	10	1

Electrical stimulation of the skin

Ether	L inn thigh	Strong induction shocks	8	5	0
Chloroform	L inn thigh	Strong induction shocks	4	2	0
Brain compression	L inn thigh	Strong induction shocks	4	2	0
Total			16	9	0

As is clear from the tables, almost every stimulation in this series, produced a reflex fall of blood-pressure, and no significant qualitative difference is observable either with different modes of stimulation or with different methods of anaesthesia or with different portions of the skin

That this statement is applicable almost without any modification to the results of stimulation of muscles and intestine will immediately be understood from the tables on page 373

Thus it is fairly clear that the stimulation of nerve terminals in the skin, muscles, and the intestine produces usually a reflex fall of blood-pressure, as was reported by Vincent and Cameron

But the threshold of stimulation for the nerve-terminals in the skin is very much higher than that for the exposed nerve-trunks Thus a stimulus which is to be reckoned a strong one for the exposed nerve-trunk is to be considered a weak one for the surface of this skin This fact explains the previously described results So far the effects have all been those of a weak stimulation, namely, a fall of the blood-pressure

If, now, we take steps to secure a considerably greater amount of stimulation by simultaneous scratching of large areas in different regions, it is not difficult to satisfy oneself that the same general law applies for the nerve-terminals as for one exposed nerve-trunk Thus, if we scratch a limited area with a moderate degree of vigor, we get a fall, while more violent application of the instruments to a large area, will give a rise (fig 19)

In the last section we compared the effects of stimulating two nerves of the same category but of different sizes, and showed that the nerves of greater size usually surpass those of smaller size in their power of evoking vasomotor reflexes, and referred to Vincent and Cameron's hypothesis that the number of afferent nerve fibers is an important factor In our stimulation of nerve endings, as a rule, we could only apply the stimulations to a small portion of the surface Now the nerve fibers spread widely from the nerve trunk, and the stimulation of a nerve would be equivalent to the stimulation of the entire surface to which the nerve is distributed In other words, the stimulation of a small portion, e g , of the skin, corresponds to that of a

Mechanical stimulation of muscles

ANESTHETIC	STIMULATED MUSCLE	MODE OF STIMULATION	REFLEX CHANGE OF BLOOD PRESSURE		
			No effect	Fall	Rise
Ether	R add mag	Scratching	0	1	0
	R sartor	Scratching	0	2	0
	R sartor	Kneading	0	3	0
	L semitend	Scratching	3	0	0
Chloroform	R add mag or l emitend	Scratching	4	0	0
Total			7	10	0

Stimulation of the intestine

Ether	Small intestine	Kneading	0	3	0
	Interior surface of small intestine	Induction shocks	1	1	0
	Interior surface of small intestine	Pinching	0	1	0
	Interior surface of small intestine	Boiling water applied	1	1	0
	Small intestine	Distension	0	4	0
Chloroform	Small intestine	Kneading	0	2	1
Brain compression	Small intestine	Kneading	0	4	0
	Interior surface of small intestine	Induction shocks	0	1	2
	Interior surface of small intestine	Scratching	0	1	0
	Interior surface of small intestine	Boiling water applied	0	1	0
	Interior surface of small intestine	Ice piece applied	0	1	0
Total			2	20	3

PLATE 1

EXPLANATION OF FIGURES -

Fig 1 The effect of increased respiratory movements upon vasomotor reflexes Bitch 9 kilos 10/5/1918 Ether The left sciatic nerve was stimulated at intervals, the stimulus increasing from left to right Upper curve, respiratory movements Lower curve, blood-pressure Base line is that of zero pressure, with periods of stimulation The height of the blood-pressure in mm Hg is indicated by the cm measured out and numbered Time in seconds For further explanation see text

Fig 2 Increased respiratory movements prevented by very deep narcosis Same bitch as in figure 1 For explanation see text

Fig 3 Effect of increased respiratory movements upon blood-pressure Bitch 14 kilos 30/5/1918 Ether Thorax intact The left sciatic nerve was stimulated The result is a marked fall of blood-pressure

Fig 4 Effect of increased respiratory movements upon blood-pressure prevented by opening the thorax Same bitch as in figure 3 Thorax wide open in the middle line The same nerve was stimulated with the same strength of stimulus as in figure 3 The result is a marked rise of blood-pressure

PLATE 3

EXPLANATION OF FIGURES

Fig 12 Scratching of the skin Dog 12 kilos 9/5/1918 Ether A marked fall of blood-pressure Respiratory movements practically unaffected

Fig 13 Application of heat (boiling water) on the skin Dog 10 kilos 25/10/1918 Brain compression and artificial respiration A fairly marked fall of blood-pressure

Fig 14 Electrical (strong) stimulation of the skin Dog 11 kilos 14/5/1918 Ether A very marked fall of blood-pressure Respiratory movements affected very slightly

Fig 15 Scratching of muscle (right sartorius) Bitch 10 kilos 22/10/1918 Ether A fairly marked fall of blood-pressure Respiratory movements show no increase

Fig 16 Kneading of muscles (lateral muscles of the right thigh) Dog 10 kilos 25/10/1918 Brain compression and artificial respiration Gentle kneading produced a pure fall (left) and violent kneading a fall followed by a rise (right) Artificial respiratory movements affected mechanically by the manipulation

Fig 17 Kneading of the small intestine Same dog as in figure 16 A fairly marked fall of blood-pressure Artificial respiratory movements slightly affected mechanically by the manipulation

Fig 18 Simultaneous tracings of the carotid blood-pressure and the volumes of kidney and hind limb Dog 10 kilos 13/12/1918 Morphia and curare Artificial respiration Strong stimulation of the right sciatic nerve caused vascular constriction of the kidney, dilatation of the limb, and a rise of blood-pressure

Fig 19 Dog 10 kilos Ether Effects of weak, moderate, and very strong stimulation of the skin The weak stimulation gives a pure fall, the moderate stimulation a rise followed by a fall while one very strong stimulation gives a pure rise

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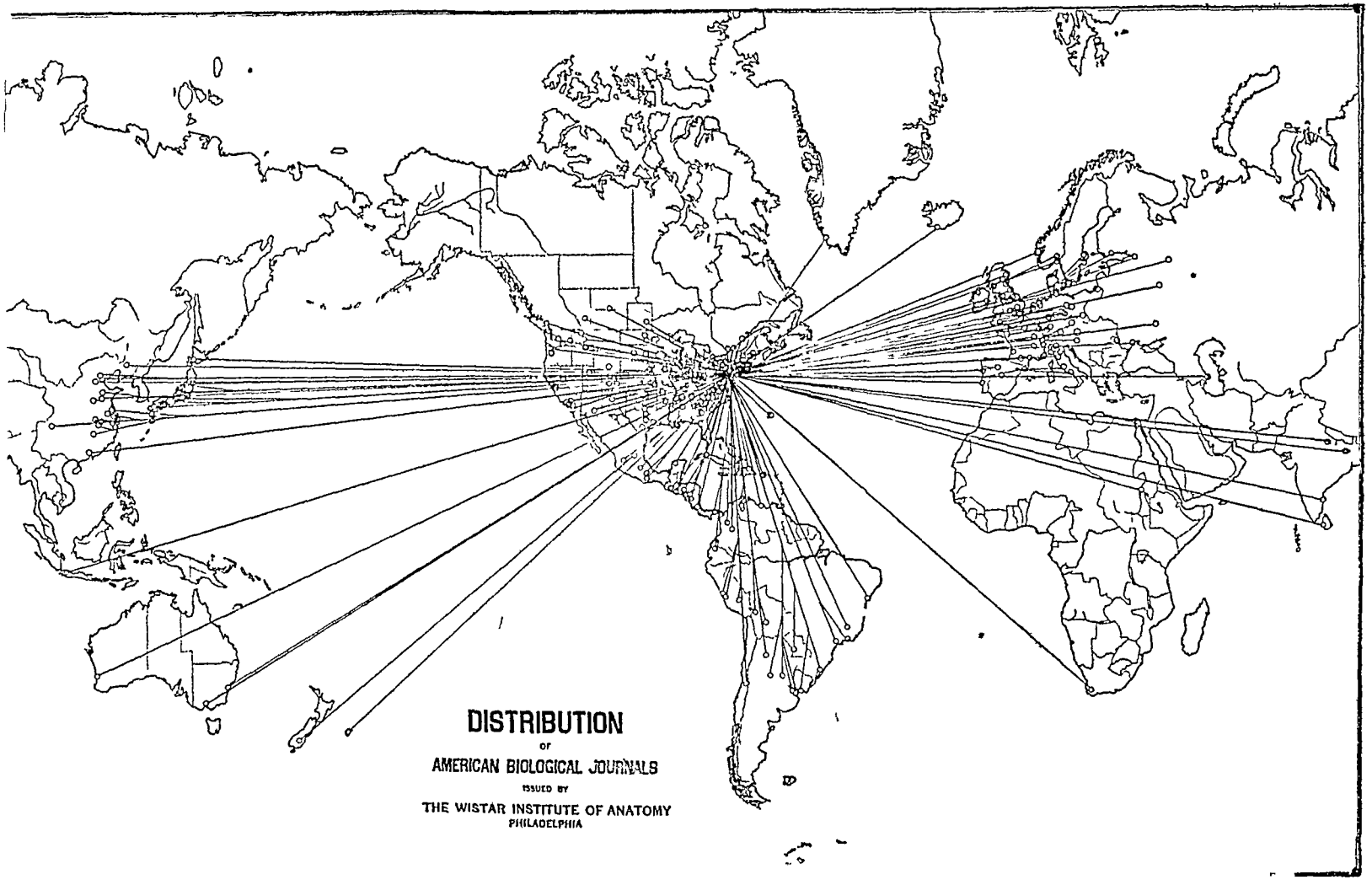
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COMPARATIVE STUDIES ON THE GROWTH OF THE CEREBRAL CORTEX

III ON THE SIZE AND SHAPE OF THE CEREBRUM IN THE NORWAY RAT (MUS NORVEGICUS) AND A COMPARISON OF THESE WITH THE CORRESPONDING CHARACTERS IN THE ALBINO RAT

NAOKI SUGITA

The Wistar Institute of Anatomy and Biology

TWO FIGURES AND TWO CHARTS

In connection with an earlier study on the size and shape of the cerebrum in the albino rat (Sugita, '17) I took up a study of the changes in the size and shape of the cerebrum in the Norway rat during its growth The method of investigation which was adopted by me for the albino rat, was followed in this case also, so that for these methods it is only necessary to refer to the paper just cited

Table 1 shows the body and the brain measurements of the Norway rats which were used The individuals have been grouped according to their brain weights and the average measurements for each group are given in the table To distinguish these from the like groups for the albino rat, which will often be referred to for comparison, a capital letter N was attached to every Norway rat group number A large part of this material has been used for further studies on cortical development or for other purposes In a subsequent paper the individual data will be presented, so that the average values alone are here printed

The material, consisting of 62 Norway rats (13 males and 19 females) whose brain weights fall between 1 1 grams and 2 4 grams was collected from time to time in the city and vicinity of Philadelphia from April to November, 1916

Figures 1 and 2 show the dorsal and lateral views of the Norway rat brain, on which the positions of the five diameters to be

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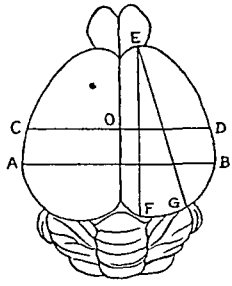
TABLE 1

Giving average values of the physical measurements for a series of Norway rats arranged according to brain weight groups Sexes combined

BRAIN WEIGHT GROUP	NUMBER OF CASES	BODY WEIGHT	BODY LENGTH	TAIL LENGTH	BRAIN WEIGHT
		grams	*mm*	*mm*	*grams*
N XI	9	19 7	86	47	1 161
N XII	0				
N XIII	2	35 5	109	87	1 356
N XIV	12	37 0	113	90	1 436
N XV	5	52 6	126	104	1 536
N XVI	8	65 8	136	118	1 644
N XVII	8	93 8	157	129	1 743
N XVIII	4	131 9	166	148	1 836
N XIX	3	193 1	187	164	1 965
N XX	5	250 9	212	179	2 033
N XXI	4	296 1	223	189	2 164
N XXII	1	336 8	223	182	2 266
N XXIII	1	394 0	256	202	2 345

measured are designated The dimensions of the figures are in accordance with the data given in table 2 and the figures are comparable with figures 1 and 2 given in the study on the albino rat brain (Sugita, '17)

Table 2 shows the average values of the five diameters of the Norway cerebrum, measured at the same locations as in the case



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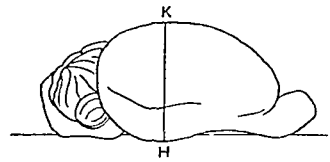

2

Fig 1 Dorsal view of the Norway rat brain weighing 1 64 grams—enlarged 1 8 diameters To show the positions at which the two measurements for the width and the two measurements for the length were taken

Fig 2 Lateral view of the Norway rat brain weighing 1 64 grams—enlarged 1 8 diameters To show the position at which the height was measured

TABLE 2

Giving the brain weight for each brain weight group the cube root of the brain weight and the linear measurements for width length and height of the cerebrum Norway rat W B = Width WB W D = Width CD L C = Length FC L F = Length FF (see figure 1) Ht = Height HK (see figure 2)

BRAIN WEIGHT GROUP	NUMBER OF CASES	BRAIN WEIGHT	CUBE ROOT OF THE BRAIN WEIGHT	W B	W D	L G	L F	Ht
		gr m		mm	mm	mm	mm	mm
N XI	9	1 161	1 051	13 86	12 69	12 63	12 18	8 83
N XII	0							
N XIII	2	1 306	1 107	14 20	13 08	13 60	13 05	9 45
N XIV	12	1 436	1 128	14 45	13 21	13 71	13 24	9 31
N XV	5	1 536	1 151	14 77	13 39	14 18	13 48	9 23
N XVI	8	1 644	1 180	14 91	13 65	14 24	13 62	9 61
N XVII	8	1 743	1 204	15 10	13 92	14 54	13 88	9 97
N XVIII	4	1 836	1 225	15 17	14 00	15 00	14 32	9 98
N XIX	3	1 965	1 252	15 68	14 28	15 93	14 65	10 08
N XX	5	2 033	1 267	15 70	14 34	15 52	14 74	10 02
N XXI	1	2 164	1 293	16 25	14 94	15 92	15 12	10 18
N XXII	1	2 266	1 314	16 60	14 90	16 15	15 70	10 35
N XXIII	1	2 345	1 329	16 55	15 65	16 30	15 90	10 25

of the albino rat and denoted by the same abbreviations (figs 1 and 2) The data are arranged in groups according to the increasing values of the brain weight at intervals of 0 1 gram

Chart 1 gives a graphic view of the average measurements of the Norway cerebrum in each brain weight group plotted on the basis of the data in table 2

A study of the individual records used for table 2 shows that within any group the individual variability does not amount to more than ±1 2 per cent, as compared with the average values for the group and each diameter shows a relatively steady increase, generally in close relation with brain weight

On examining Chart 1, the curves for *W B* and *W D* are found to run almost parallel and the same is true for the curves *I G* and *L F* as seen already in the case of the Albino cerebrum By a comparison of the graphs for the width with the graphs for the length it is evident that the rapidity of growth along the sagittal diameter is greater than that along the frontal diameter a relation that was also seen in the Albino cerebrum *Ht* increases slowly as compared with the other diameter

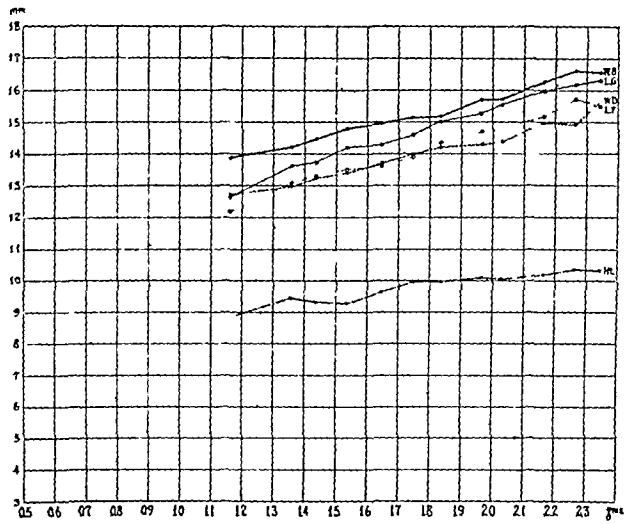

Chart 1 Giving the average values of the five diameters of the Norway r cerebrum for each brain weight group

WB WD LG LF Ht

The approximate value of each diameter can be calculated the following formulas

$$W\ B\ (\text{mm}) = C_W \times \sqrt[3]{\text{Brain weight (grams)}}$$

where C_W will be 12 8 for a brain weighing 1 3–1 6 grams
12 5 for a brain weighing 1 6–2 4 grams

$$W\ D\ (\text{mm}) = W\ B\ (\text{mm}) - 1\ 25\ \text{mm}$$

$$L\ G\ (\text{mm}) = C_L \times \sqrt[3]{\text{Brain weight (grams)}}$$

where C_L will be 12 2 for any brain weighing 1 3–2 4 grams

$$L\ F\ (\text{mm}) = L\ G\ (\text{mm}) \times 0\ 955$$

$$Ht\ (\text{mm}) = C_H \times \sqrt[3]{\text{Brain weight (grams)}}$$

where C_H will be 8 5 for a brain weighing 1 1–1 4 grams
8 2 for a brain weighing 1 4–1 9 grams
7 9 for a brain weighing 1 9–2 4 grams

At the first entry in this study, Group N XI (table 2), relative volume of the cerebrum ($W\ B \times L\ G \times Ht$) is

$$13\ 86 \times 12\ 63 \times 8\ 83 = 1546\ 92$$ (see also table 3 A)

corresponding to a brain weighing 1 161 grams (body weight 19 7 grams, body length 86 mm , tail length 47 mm and age estimated at about 10 days), while the relative volume of a cerebrum in the last entry, Group N XXIII (table 2), is

16 55 × 16 30 × 10 25 = 2765 09 (see also table 3 A)

corresponding to a brain weighing 2 345 grams (body weight 394

TABLE 3

Giving the relative cerebral volumes obtained by the formula W B × L G × H t, for each brain weight group of the Norway (A) and of the Albino (B) The albino rat brain weight corresponding to the given Norway brain of the same age obtained by reducing the Norway brain weight by 18 per cent is also given in (1)

(A) NORWAY RAT				(B) ALBINO RAT		
Brain weight group N XI–N XXIII	Observed brain weight Norway rat	Calculated weight of the Albino brain of the same age	Relative volume of the cerebrum of Norway rat obtained by the formula W B × L G × H t, based on data in table 2	Brain weight group IX–XX	Observed brain weight Albino rat	Relative volume of the Albino cerebrum obtained by the formula W B × L G × H t, based on data in table 3 (Sugita, 17)
	grams	grams			grams	
N XI	1 161	0 952	1017	IX	0 934	1330
N XIII	1 356	1 112	1870	X	1 017	1396
N XIV	1 436	1 177	1844	XI	1 108	1522
N XV	1 536	1 259	1944	XII	1 203	1606
N XVI	1 644	1 348	2040	XIII	1 334	1663
N XVII	1 743	1 429	2189	XIV	1 449	1788
N XVIII	1 836	1 505	2271	XV	1 598	1956
N XIX	1 962	1 612	2407	XVI	1 662	2014
N XX	2 033	1 667	2419	XVII	1 737	2157
N XXI	2 164	1 774	2634	XVIII	1 832	2228
N XXII	2 266	1 858	2776	XIX	1 924	2286
N XXIII	2 345	1 923	2765	XX	2 037	2468

grams, body length 256 mm and tail length 202 mm) According to these determinations, the volume increases by 79 per cent while the weight increases by 102 per cent showing roughly that the specific gravity of a brain in the fully mature Norway rat is higher than that of younger one

By an estimate based on the data given by Donaldson and Hatai ('11), it would appear that if the Albino and the Norway

brains of the same age be compared, the Norway brain weight is 20 to 25 per cent higher than the albino brain weight, when the albino brain weight is taken as the standard For the purpose of comparison in their developmental stages, I have assumed that the Norway brain would correspond to an Albino brain whose weight is 18 per cent less than the Norway brain weight of like age Here the Norway brain weight is taken as the standard The evidence for this conclusion will be given in detail in a later paper which discusses the thickness of the cortex in the brain of the Norway rat

A comparison of the Norway brain with that of the Albino may be made in two ways, by a comparison of brains of like weight or by a comparison of brains of like ages In Chart 2, the diameters of the cerebrum in the Norway rat are compared with those in the albino rat In part A of this chart, the data for the Norway and the albino rats were entered according to the observed brain weights, and in part B of the same chart, the same linear measurements for the Norway as used in part A are entered above brain weights which are 18 per cent below the observed Norway brain weights and which in turn represents the weights for the albino brains of like age with those of the Norway rat The corresponding brain weights of the Norway and of the albino rats at the same age are given in table 3 A It is assumed that the albino brain weight is 82 per cent of that for the Norway

If, as shown in part A of Chart 2, the comparison is made between the brains of the two rats using similar brain weight groups, *W B* in the Norway cerebrum surpasses *W B* in the Albino on the average by 0 4 mm *L G* is quite equal in both the rats for brains weighing 1 1 to 1 6 gms, after which stage it is clearly greater for the Albino *Ht* is on the average slightly in favor of the Norway

If, on the other hand, as shown in part B of the chart, a Norway cerebrum be compared with an Albino cerebrum of the same age (over 10 days), the Norway cerebrum has a greater *W B* than the Albino, by about 1 00 mm , and also a greater *L G* The excess of *L G* in the early age is on the average 0 7 mm , but this difference decreases as the age advances, owing to the more rapid growth of the albino cerebrum in this dimension

Ht in the Norway cerebrum is greater on the average than that of the Albino of the same age by ca 0 6 mm As was to be expected the excess in the dimensions of the Norway brain are greater in part B, Chart 2, where the brains are compared according to age, because at like ages the Norway brain is heavier

The chief point of interest brought out by this comparison is the similarity in the direction of the corresponding curves for the two forms and the fact that the age at which *L G* crosses *W B* in the Albino is approximately the age at which these diameters come nearest to crossing in the Norway

Table 3 A gives the relative volumes of the cerebrum in each brain weight group of the Norway rat, obtained by the formula

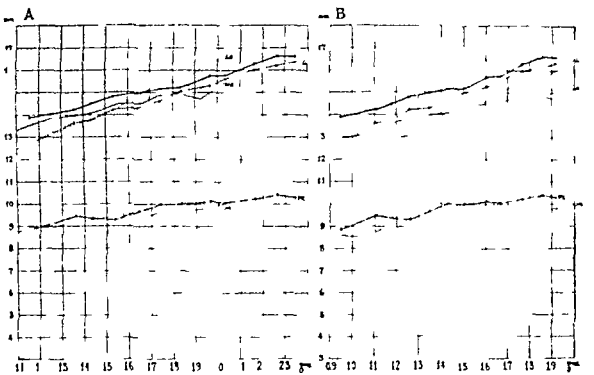

Chart 2 Giving a comparison of the cerebral measurements (*W B I G* and *Ht*) of the Norway and the albino rats Part A was plotted according to the observed weight of the brain for each brain weight group of the Norway rat Part B was plotted according to the corresponding weight of the brain in the Albino of the same age the Albino brain weights being obtained by reducing the observed weight of the Norway brain by 18 per cent The measurements for the Norway cerebrum were based on table 2 of this paper and the measurements for the Albino cerebrum were based on table 3 of the first paper of this series (Sugita 17)

W B (Norway) W B (Albino)
L G (") L G (")
Ht (") Ht (")

$W\ B \times L\ G \times Ht$, based on the measurements given in table 2 Table 3 B gives the similar calculations for the Albino cerebrum based on the measurements given in table 3 of the first paper in this series (Sugita, '17)

Table 4 gives for both the rats the cerebral volumes (A) according to brain weight, and (B) according to age In table 4 A,

TABLE 4

Giving the ratios of the cerebral volumes of the Norway to the Albino rats (A) pairing the brains of the same weight and (B) pairing the brains of the same age Calculated on the basis of the data given in table 3

(A)			(B)		
Pairs of groups in which a comparison of cerebral volume was made Like brain weights		Ratio of cerebral volume between two groups of the like weight Albino = 1 000	Pairs of groups in which a comparison of cerebral volume was made Like ages		Ratio of cerebral volume between two groups of the like age Albino = 1 000
Norway	Albino		Norway	Albino	
N XI	XI	1 016	N XI	IX	1 159
N XIII	XIII	1 097	N XIII	X[1]	1 271[1]
N XIV	XIV	1 031	N XIV	XI	1 212
N XV	XV	0 994	N XV	XII	1 210
N XVI	XVI	1 013	N XVI	XIII	1 227
N XVII	XVII	1 015	N XVII	XIV	1 224
N XVIII	XVIII	1 019	N XVIII	XV	1 161
N XIX	XIX	1 053	N XIX		
N XX	XX	0 951	N XX	XVI	1 212
			N XXI	XVII	1 221
			N XXII	XVIII	1 248
			N XXIII	XIX	1 210
Average		1 021	Average		1 214

[1] As the reduced brain weight of Group N XIII falls between the brain weights of Groups X and XI, the mean value of the cerebral volumes of Groups X and XI was used in comparison

the brains of like brain weight groups are compared, by pairing the groups which carry the same number By this comparison, it is seen that, on the average, the values for the Norway rat are somewhat greater, except in Group XV and in the old age group, Group XX, in which the reverse is true This shows that the Norway brain has, as a rule, a less specific gravity than the brain of the albino which has the same weight One important

factor in producing this relation is that the Norway brain is younger and less advanced in myelination than the albino brain of the same weight

In table 4 B the cerebral volume of a Norway rat is compared with the cerebral volume of an Albino of presumptively the same age Each Norway brain weight group is paired with an albino group which has the average brain weight nearest to the corresponding albino brain weight of the same age with the Norway group The data were all taken from table 3 Compared in this way, the Norway cerebrum has a volume about 21 per cent above that of the albino cerebrum of the same age, as shown in table 4 B

The width-length index of the Norway cerebrum, which is obtained according to formula $\frac{W \quad D \times 100}{L \quad F}$, is 104 in the youngest Group N XI, and decreases as the brain weight advances, dropping to 97 or less in the last and oldest groups Compared with the like group of the Albino, the width length index of the Norway cerebrum is on the average always higher by 2 or more points than that of the albino cerebrum So, it may be concluded that the Norway cerebrum is becoming somewhat elongated as the age advances, but not to so marked a degree as does the albino cerebrum and that it is always somewhat more rounded in shape as compared with the albino cerebrum of the same weight or age The method of measurement here used reveals only in part the degree of difference in the shape of the two brains, for direct inspection shows the surface of the Norway cerebrum to be distinctly more rounded than that of the Albino, especially at the frontal poles

LITERATURE CITED

DONALDSON H H AND HATAI S 1911 A comparison of the Norway rat with the albino rat in respect to body length brain weight spinal cord weight and the percentage of water in both the brain and the spinal cord Jour Comp Neur vol 21 pp 417–458

SUGITA NAOKI 1917 Comparative studies on the growth of the cerebral cortex I On the changes in the size and shape of the cerebrum during the growth of the brain Albino rat Jour Comp Neur vol 28 pp 493–510

differences in the cerebral cortex. Consequently, it became desirable for me to compare in these two forms, one wild and aggressive and the other gentle and domesticated, the course of the growth of the cerebral cortex.

Using my previous studies on the Albino (Sugita, '17a) and on the form of the Norway brain (Sugita, '18) as a point of departure, I will present in this paper the data on the cortical thickness of the Norway rat and will compare these with the data for the Albino. In this comparison of the brains of the two forms, the data, other than those on cortical thickness, are all quoted from Donaldson and Hatai ('11, '15).

II. MATERIAL

The Norway rats used in this study, were all supplied through the courtesy of The Wistar Institute and were trapped alive in Philadelphia and its vicinity, between April and November, 1916. There were 36 males and 18 females, representing every stage of growth between 17 and 394 grams in body weight. In the preparation of the material and the arrangement of the data, the same methods as those described in my former study on the Albino (Sugita, '17a) were followed. For the discrimination of a Norway group from an albino group of the same tabular number, the Norway records carry the capital letter N before their group number.

The following tables, tables 1 and 2, give the sex, body and tail lengths, and body and brain weights of the Norway rats used in this study, grouped according to their brain weights and averaged for each group. Table 1 contains the material used for the sagittal and frontal sections and table 2 that for the horizontal sections.

Comparing the body measurements of this series with those given in table 85 in "The Rat" (Donaldson, '15), it is found that the average values for my material by groups correspond fairly well with the table values.

The increase in the body measurements of the Norway rat according to age is imperfectly known, so that we can not infer the age from the body measurements with any exactness

TABLE 1

Showing the sex body weight and length tail length and brain weight of the Norway rats used in this study (sagittal and frontal sections) accompanied by the averages for each brain weight group

NO	LITTER NO	SEX	BODY WEIGHT	BODY LENGTH	TAIL LENGTH	BRAIN WEIGHT
			gram	*mm*	*mm*	*gr m*
XI b	(1)	m	19 8	84	41	1 150
a	(1)	m	20 8	86	44	1 160
i	(2)	m	17 8	85	65	1 175
			19 5	*85*	*50*	*1 164*
XII						
XIII a	(3)	m	35 3	110	66	1 369
			35 3	*110*	*86*	*1 369*
XIV b	(4)	m	33 1	104	84	1 407
g	(3)	m	37 5	112	88	1 420
a	(4)	m	33 8	113	91	1 431
i	(3)	m	36 3	107	86	1 431
e	(5)	m	43 6	121	108	1 437
k	(3)	f	36 1	112	87	1 440
			36 7	*112*	*91*	*1 430*
XV e		m	42 6	122	102	1 517
e		m	66 7	135	114	1 557
			54 7	*129*	*108*	*1 537*
XVI a		m	71 8	137		1 619
g		m	54 8	130	107	1 632
e		m	56 3	128	105	1 636
			60 0	*132*	*106*	*1 629*
XVII e		f	81 0	152	120	1 710
g		m	57 0	137	113	1 721
a		f	118 0	172	136	1 738
c		f	104 0	161	132	1 788
			90 1	*156*	*125*	*1 739*
XVIII c		f	136 9	157	147	1 820
a		m	128 1	177	112	1 833
			132 5	*167*	*145*	*1 829*
XIX b		m	160 7	177	158	1 962
a		f	201 0	210	174	1 981
			205 9	*194*	*166*	*1 972*

On the basis of these rough data, the approximate age of the individuals in tables 1 and 2 can be inferred

To my regret, I did not obtain material under 17 grams in body weight I could, therefore, not make a complete study of the postnatal growth of the cerebral cortex of the Norway rat from birth on and must in consequence be content to present in this paper the data beginning with material probably from 10 to 12 days old It may, however, be noted here, that, among the rats trapped, the following were evidently members of the same litter and still following the mother

(1) N XI a, N XI b, N XI c, N XI d, with their mother N XX a, and three other young which were used for another purpose

(2) N XI i, N XI h, and four others

(3) N XIII a, N XIII b, N XIV g, N XIV h, N XIV i, N XIV j, N XIV k

(4) N XIV e, N XIV f, and two others

(5) N XIV a, N XIV b, N XIV c, N XIV d and two others, with their mother N XX b

This suggests that the Norway rats whose brain weighs less than 1 5 grams or whose body weighs less than about 40 grams are not yet independent of their mothers

III TECHNIQUE

For the technique of fixation and imbedding and the making and staining of the sections, the same procedures which have been already described (Sugita, '17 a) were followed Thirteen different localities were measured on sections in three planes corresponding to those used in the former study of the Albino cortex (cf figs 2, 4 and 6 in the paper cited)

As to the cortical cell-lamination of the Norway rat, two sets of figures with explanations were given in the former paper (Sugita, '17 a) reproduced from Lewis (1881) and Fortuyn ('14) and to those I would like to call attention on this occasion There does not appear to be any important difference between the Norway and the albino rats in the cell-lamination of the cerebral cortex

IV OBSERVED DATA GIVEN IN TABLE AND CHART

As in the case of the albino rat the measurement of the cortical thickness of the Norway brain was made at the localities I–XIII by the direct measurement of the sections as prepared and was then recorded without correction The results thus obtained are condensed in table 3

Table 3 shows for each brain weight group the average thickness of the cerebral cortex of the Norway rat as directly observed in each of the three sections and the general average obtained by averaging the thicknesses of the sagittal, frontal and horizontal sections The average brain weight corresponding to the average thickness of the cortex is obtained by doubling the weight of the brain, from which the sagittal and frontal sections were taken, adding the weight of the brain from which the horizontal sections were taken, and dividing the sum by three

Chart 1 is based on table 3 and shows the increase in the general average thickness of the cerebral cortex of the Norway rat,

TABLE 3

Showing the general average thickness of the cerebral cortex of the Norway rat according to brain weight groups also the average thickness in the sagittal frontal and horizontal sections Observations on slide without correction

BRAIN WEIGHT GROUP	SAGITTAL SECTION		FRONTAL SECTION	HORIZONTAL SECTION			GENERAL AVERAGE	
	Number of cases	Brain weight	Thickness	Thickness	Number of cases	Brain weight	Thickness	Brain weight	Thickness
		gr m	mm	mm		gr m	mm	gr m	mm
N XI	3	1 164	1 34	1 43	3	1 164	1 44	1 164	1 40
N XII	0				0				
N XIII	1	1 369	1 35	1 50	1	1 343	1 50	1 360	1 45
N XIV	6	1 430	1 43	1 48	5	1 447	1 54	1 436	1 48
N XV	2	1 53[illegible]	1 10	1 50	2	1 5[illegible]0	1 58	1 532	1 49
N XVI	3	1 629	1 41	1 54	4	1 663	1 63	1 640	1 53
N XVII	4	1 739	1 51	1 56	4	1 747	1 59	1 742	1 55
N XVIII	2	1 899	1 51	1 64	2	1 843	1 63	1 834	1 59
N XIX	2	1 972	1 56	1 58	1	1 963	1 68	1 964	1 61
N XX	2	2 052	1 49	1 48	2	2 018	1 58	2 041	1 52
N XXI	2	2 172	1 53	1 53	2	2 150	1 7	2 166	1 60
N XXII	0				0				
N XXIII	1	2 31[illegible]	1 60		1	2 31[illegible]	1 73	2 31[illegible]	1 67

TABLE 4—Continued

BRAIN WEIGHT GROUP	BRAIN WEIGHT	CORRECTION COEFFICIENT		THICKNESS OF THE CORTEX (SAGITTAL SECTION)					
		Diam L F on fresh brain	Diam L F on slide	Loc I	Loc II	Loc III	Loc IV	Loc V	Average
	grams	*mm*	*mm*	*mm*	*mm*	*mm*	*mm*	*mm*	*mm*
N XIX b	1 962	14 70	11 50	2 98	2 12	1 97	1 54	1 46	2 01
a	1 981	14 40	11 50	2 83	2 08	1 75	1 54	1 44	1 93
	1 972	*1 26*		*2 91*	*2 10*	*1 86*	*1 54*	*1 45*	*1 97*
N XX c	2 015	14 55	11 50	2 86	2 00	1 81	1 55	1 43	1 93
a	2 089	14 95	12 00	2 75	1 93	1 67	1 34	1 30	1 80
	2 052	*1 25*		*2 81*	*1 97*	*1 74*	*1 45*	*1 37*	*1 87*
N XXI g	2 156	15 15	11 90	3 01	2 15	1 82	1 58	1 40	1 99
d	2 187	15 30	11 50	2 94	2 09	1 85	1 60	1 41	1 98
	2 172	*1 30*		*2 98*	*2 12*	*1 84*	*1 59*	*1 41*	*1 99*
N XXII									
N XXIII a	2 345	14 50	12 50	2 74	2 07	1 75	1 38	1 33	1 86
	2 345	*1 16*		*2 74*	*2 07*	*1 75*	*1 38*	*1 33*	*1 86*

values shown in table 3 This table (table 7) serves as a standard for discussing the actual thickness of the fresh cortex of the Norway rat The average thickness in the adult Norway rat is 2 06 mm , as obtained by averaging the thicknesses of the cortex in Groups N XV–N XXIII, in which stages the cortex may be considered to have reached its full thickness

Charts 2 to 7 show graphically the data given in tables 4 to 6 respectively, and chart 8, which is based on table 7 giving the average values, presents a general picture of the growth changes in the cortex according to brain weight

Charts 2, 4 and 6 show the individual determinations for the thickness of the cortex in the sagittal, frontal and horizontal sections, respectively, plotted according to the brain weight Chart 2 gives the individual records for locality I and locality V with the average for all localities from I to V in the sagittal sections In a like manner, chart 4 gives the values for localities VII and VIII with the average of localities VI to VIII for the

TABLE 5

Showing the corrected values of the cortical thickness in the frontal section for each individual and for each brain weight group The data for the correction-coefficients are indicated separately for each brain and the coefficient is given with the average for each group

BRAIN WEIGHT GROUP	BRAIN WEIGHT	COEFFICIENT		THICKNESS OF THE CORTEX (FRONTAL SECTION)			
		Diam W D on fresh brain	Diam W D on 1 le	Loc. VI	Loc. VII	Loc VIII	Average
	gram	mm.	mm	mm	mm	mm	mm.
N XI b	1 155	13 00	9 90	2 05	2 11	1 68	1 95
a	1 160	12 70	10 00	1 94	1 92	1 62	1 83
i	1 175	12 50	9 20	1 96	1 99	1 62	1 86
	1 164	*1 31*		*1 98*	*2 01*	*1 64*	*1 88*
N XII							
N XIII a	1 369	13 00	10 00	2 07	2 21	1 59	1 96
	1 369	*1 30*		*2 07*	*2 21*	*1 59*	*1 96*
N XIV b	1 107	13 05	9 90	2 27	2 13	1 71	2 04
g	1 129	13 20	9 50	2 18	2 29	1 71	2 06
a	1 431	12 80	10 70	2 04	2 00	1 73	1 92
i	1 431	13 40	10 30	1 90	2 06	1 67	1 84
e	1 437	13 20	9 80	1 89	2 13	1 56	1 86
k	1 440	13 30	9 90	2 12	2 10	1 65	1 97
	1 430	*1 32*		*2 07*	*2 18*	*1 66*	*1 95*
N XV e	1 517	13 20	10 00	1 98	2 21	1 62	1 94
c	1 557	13 00	9 60	2 28	2 39	1 76	2 14
	1 537	*1 36*		*2 13*	*2 30*	*1 69*	*2 04*
N XVI a	1 619	13 80	10 80	2 01	2 13	1 72	1 95
g	1 632	13 70	9 90	2 21	2 37	1 83	2 21
e	1 636	13 80	10 00	2 14	2 30	1 76	2 08
	1 629	*1 35*		*2 13*	*2 35*	*1 77*	*2 08*
N XVII e	1 710	13 80	10 00	2 15	2 31	1 68	2 05
g	1 721	13 60	10 40	2 20	2 35	1 75	2 10
a	1 738	14 10	10 60	2 01	2 17	1 66	1 95
c	1 788	13 95	10 60	2 35	2 40	1 82	2 19
	1 739	*1 33*		*2 18*	*2 31*	*1 73*	*2 07*
N XVIII e	1 820	14 45	10 70	2 20	2 35	1 73	2 09
a	1 833	13 05	11 70	2 18	2 25	1 80	2 07
	1 8 9	*1 27*		*2 19*	*2 29*	*1 77*	*2 08*

TABLE 5—Concluded

BRAIN WEIGHT GROUP	BRAIN WEIGHT	COEFFICIENT		THICKNESS OF THE CORTEX (FRONTAL SECTION)			
		Diam H D on fresh brain	Diam H D on slide	Loc VI	Loc VII	Loc VIII	Average
	grams	*mm*	*mm*	*mm*	*mm*	*mm*	*mm*
N XIX b	1 962	14 60	11 60	2 08	2 28	1 68	2 01
a	1 981	13 95	10 80	2 04	2 21	1 72	1 99
	1 972	*1 26*		*2 06*	*2 25*	*1 70*	*2 00*
N XX c	2 015	14 30	10 50	2 14	2 32	1 73	2 06
a	2 089	14 50	11 20	1 80	2 08	1 66	1 85
	2 052	*1 33*		*1 97*	*2 20*	*1 70*	*1 96*
N XXI g	2 156	14 75	11 10	2 03	2 24	1 69	1 99
d	2 187	15 05	10 80	2 19	2 54	1 77	2 17
	2 172	*1 36*		*2 11*	*2 39*	*1 73*	*2 08*
N XXII							
N XXIII							

frontal sections Chart 6 does the same for localities IX and XIII with the average of localities IX to XIII in the horizontal sections Charts 3, 5 and 7 show the average values of the cortical thickness in the sagittal, frontal and horizontal sections, for each brain weight group Further, on each chart is shown the change in thickness at each one of the localities measured in that section

Chart 8 is based on table 7 and shows the general average (corrected) thickness of the cerebral cortex of the Norway rat according to the brain weight and also the average thickness in each of the sections

VI DISCUSSION

The relations existing between each of the several localities measured in this study of the Norway are quite similar to the relations found in the cerebral cortex of the albino rat Individual variations appear, but these are no higher than ±6 per cent, compared with the average values of the group No sex differ-

TABLE 6

Showing the corrected values of the cortical thickness in the horizontal section for each individual and for each brain weight group. The data for the correction coefficients are indicated separately for each brain and the coefficient in given with the average for each group

BRAIN WEIGHT GROUP	BRAIN WEIGHT	COEFFICIENT		THICKNESS OF THE CORTEX (HORIZONTAL SECTION)					
		Diameter Bolo fresh brain	Diameter Bolo fixed	Layer IX	Layer X	Layer VI	Layer II	Layer XIII	Average
	gm.	mm.	m.	mm.	mm.	mm.	mm.	mm.	mm.
XXI d	1 133	13 80	11 00	2 52	1 91	1 72	1 56	1 31	1 80
h	1 160	13 80	10 10	2 85	1 99	1 92	1 69	1 50	1 99
c	1 199	13 90	10 80	2 52	1 81	1 7	1 62	1 33	1 81
	1 164	*1 30*		*2 63*	*1 91*	*1 80*	*1 62*	*1 8*	*1 87*
XXII									
XXIII b	1 343	14 20	10 30	2 91	2 02	1 03	1 85	1	1 05
	1 343	*1 38*		*2 91*	*02*	*2 05*	*1 85*	*1 57*	*08*
XXIV e	1 107	14 0	10 60	2 37	2 06	1 91	1 72	1 48	1 91
h	1 1 8	14 30	10 70	3 19	2 11	2 08	1 85	1	10
j	1 413	14 35	10 10	2 96	2 22	1 1	1 95	1 09	1 21
f	1 473	14 55	10 00	1 92	2 08	1 03	1 81	1 33	1 08
d	1 181	14 60	10 30	1 91	1 22	1 88	1 71	1 6	1 06
	1 147	*1 36*		*2 91*	*° 14*	*° 03*	*1 81*	*1 57*	*° 01*
XXV b	1 511	11 6	10 10	3 19	2 39	2 15	1 97	1 78	2 28
d	1 529	14 50	10 50	3 11	1 21	2 12	1 91	1 6	2 19
	1 20	*1 41*		*3 15*	*2 27*	*2 14*	*1 94*	*1 70*	*2 24*
XXVI f	1 613	14 90	11 00	3 23	2 31	2 10	1 96	1 66	2 26
h	1 666	15 00	10 90	2 96	2 23	2 29	1 94	1 70	2 32
d	1 674	15 15	11 30	2 97	2 14	2 12	1 85	1 59	2 12
b	1 699	14 75	11 05	3 33	2 36	1 92	1 70	1 48	1 7
	1 668	*1 55*		*3 12*	*2 27*	*2 09*	*1 88*	*1 61*	*2 19*
XXVII f	1 717	15 45	11 45	2 89	2 18	2 16	1 93	1 62	2 16
h	1 718	14 60	11 20	2 73	2 17	2 01	1 80	1 41	2 03
d	1 773	15 10	10 75	2 77	2 23	2 13	1 81	1 51	2 50
h	1 779	15 40	11 35	3 27	2 31	1 18	2 02	1 72	2 30
	1 747	*1 35*		*2 92*	*2 24*	*2 13*	*1 90*	*1 57*	*2 15*
XXVIII b	1 815	15 00	11 00	3 06	2 41	2 08	1 84	1 57	2 15
d	1 870	15 40	10 85	3 60	2 38	2 14	1 98	1 76	2 37
	1 843	*1 39*		*3 33*	*2 30*	*2 11*	*1 90*	*1 67*	*2 26*

TABLE 6—Concluded

BRAIN WEIGHT GROUP	BRAIN WEIGHT	COEFFICIENT		THICKNESS OF THE CORTEX (HORIZONTAL SECTION)					
		Diam W B on fresh brain	Diam W B on slide	Loc IX	Loc X	Loc XI	Loc XII	Loc XIII	Average
	grams	*mm*	*mm*	*mm*	*mm*	*mm*	*mm*	*mm*	*mm*
N XIX c	1 953	15 65	11 70	3 14	2 17	2 14	2 01	1 81	2 25
	1 953	*1 34*		*3 14*	*2 17*	*2 14*	*2 01*	*1 81*	*2 25*
N XX e	2 008	15 55	11 90	3 10	2 28	2 09	1 87	1 52	2 17
b	2 028	15 85	12 00	2 78	2 05	1 97	1 71	1 43	1 99
	2 018	*1 31*		*2 94*	*2 17*	*2 03*	*1 79*	*1 48*	*2 08*
N XXI f	2 150	16 55	12 10	3 30	2 66	2 22	1 94	1 57	2 34
j	2 162	16 25	12 40	3 60	2 38	2 16	1 98	1 57	2 34
	2 156	*1 34*		*3 45*	*2 52*	*2 19*	*1 96*	*1 57*	*2 34*
N XXII									
N XXIII a	2 345	16 55	12 90	3 14	2 58	2 17	1 69	1 54	2 22
	2 345	*1 28*		*3 14*	*2 58*	*2 17*	*1 69*	*1 54*	*2 22*

ence in cortical thickness is recognizable when the brain weights are similar

In the sagittal sections, the cortex attains nearly its full thickness when the brain weighs 1 63 grams (Group N XVI), while in the frontal and horizontal sections, this is attained somewhat earlier, that is, in the brains weighing 1 53 grams (Group N XV) (cf charts 3, 5, 7, 8) In the general average thickness of the cortex of the Norway rat, the full thickness is attained in the brains weighing about 1 53 grams, at which phase the body weight observed is about 55 grams (tables 1 and 2) (chart 8) In the full grown Norway rat at brain weights between 1 6 and 2 4 grams, the average cortical thickness ranges between 1 97 and 2 14 mm , with a mean value for Groups N XVI–N XXIII (table 7) of 2 06 mm The average thickness for each locality is given in table 8 for the Norway rat, together with the corresponding values for the Albino, thus making it possible to compare the cortical thickness in the two forms

TABLE 7

Showing the average corrected thickness of the cerebral cortex in the Norway rat for each brain weight group

BRAIN WEIGHT GROUP	SAGITTAL SECTION		FRONTAL SECTION	HORIZONTAL SECTION		CENTRAL AVERAGE	
	Brain weight	Thickness	Thickness	Brain weight	Thickness	Brain weight	Thickness
	grams	*mm*	*mm*	*grams*	*mm*	*grams*	*mm*
NXI	1 164	1 61	1 88	1 164	1 87	1 164	1 79
NXII							
NXIII	1 369	1 73	1 96	1 343	2 08	1 360	1 92
NXIV	1 430	1 84	1 90	1 447	2 09	1 436	1 96
NXV	1 537	1 82	2 04	1 520	2 24	1 532	2 03
NXVI	1 629	1 88	2 08	1 663	2 19	1 640	2 05
NXVII	1 739	1 94	2 07	1 747	2 15	1 742	2 05
NXVIII	1 829	1 93	2 08	1 843	2 26	1 834	2 09
NXIX	1 972	1 97	2 00	1 903	2 25	1 965	2 07
NXX	2 052	1 87	1 96	2 018	2 08	2 041	1 97
NXXI	2 172	1 99	2 08	2 156	2 34	2 166	2 14
NXXII							
NXXIII	2 345	1 86		2 345	2 22	2 345	2 04

Within the limits of our material, the course of development of the cortical thickness in every locality seems in general, similar to that in the corresponding locality of the albino rat, the descriptions of which were given in the former paper (Sugita, '17 a, pp 574–577)

VII A COMPARISON OF THE NORWAY RAT WITH THE ALBINO RAT IN RESPECT OF CORTICAL THICKNESS

The main object of the present paper is to compare the data from the Norway with those from the albino rat, in respect of the cortical thickness, a comparison of much interest, since the two forms are so closely related genetically and at the same time show differences in body size and in absolute brain weight which have been already noted

Comparing the mature brains, which weigh alike, of the both forms (table 8), the Norway cortex, whose thickness on the average in Groups NXVI to NXX (brain weight average 1 844 grams) is 2 05 mm surpasses by 0 15 mm or 8 per cent the albino cortex whose thickness on the average in Groups XVI to XX

TABLE 8

A comparison of the cortical thicknesses at each locality and on the average, in the adult Norway and the albino brains of the same absolute weight The measurements used here are average values of Groups N XVI–N XX and Groups XVI–XX respectively, taken from tables 4 to 6 of this paper and tables 6 to 8 (Sugita, '17a) The corresponding brain weights are 1 844 grams in the Norway and 1 815 grams in the Albino The thickness of the Albino cortex is always taken as the standard for computing the percentage differences

SECTIONS	LOCALITIES	THICKNESS OF THE CORTEX		CORTEX OF THE NORWAY RAT EXCEEDS BY
		Norway rat	Albino rat	
		mm	*mm*	*per cent*
Sagittal	Locality I	2 84	2 80	1 4
	II	2 06	1 92	7 3
	III	1 82	1 74	4 6
	IV	1 51	1 36	10 0
	V	1 37	1 19	15 1
	Average	1 92	1 80	6 7
Frontal	Locality VI	2 11	1 84	14 8
	VII	2 28	2 18	4 6
	VIII	1 73	1 59	8 9
	Average	2 04	1 87	9 1
Horizontal	Locality IX	3 09	3 08	0 3
	X	2 23	2 06	8 2
	XI	2 10	2 04	3 0
	XII	1 90	1 71	11 1
	XIII	1 63	1 27	28 3
	Average	2 19	2 03	8 0
General average		2 05	1 90	8 0

two forms is made so as to bring those of approximately the same age on the same line of the table It will be seen by these comparisons that the Norway rat brain, if paired with the albino rat brain of like age, shows almost the same value of the percentage of water, while the brain weight differs by 16 to 20 per cent in favor of the Norway rat brain, the weight of the Norway brain being taken as the standard

So, from the point of view of age, a Norway rat brain should be in the same phase of development with an albino brain,

which weighs 16 to 20 per cent less With this relation in view, I reduced by 18 per cent—which is the mean value of 16 to 20 per cent (see table 9)—the weight of the Norway rat brains in table 7, and assumed that I thus obtained brain weights which represent the corresponding brain weights of the albino rat in respect to the cortical development I have plotted the values for the actual cortical thickness on the reduced brain weights by

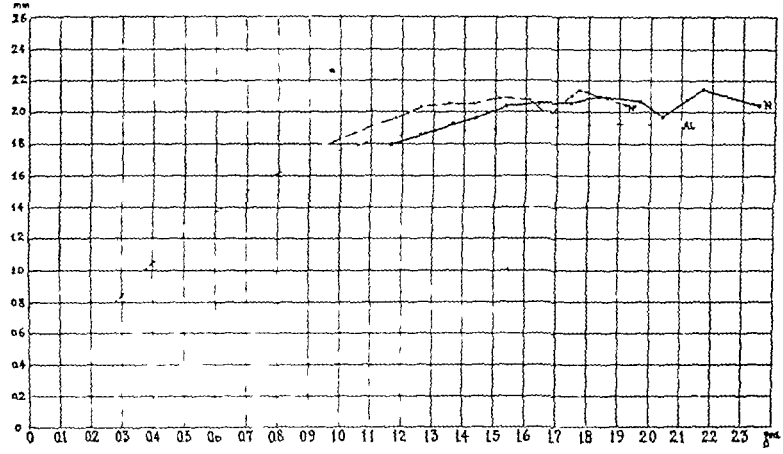

Chart 9 Giving a comparison of the thickness of the Norway cortex with that of the albino cortex, on brain weight •——•N General average thickness of the Norway cortex according to the actual brain weight group - - - - -AL General average thickness of the Albino cortex according to the brain weight group Smoothed Taken from chart 9 of the second paper of this series •—•—• N′ General average thickness of the Norway cortex entered according to the reduced brain weight representing the albino brain weight of the corresponding age

the dot and dash line in chart 9, in which the smoothed graph for the cortical thickness of the albino rat is represented by a dotted line Glancing at the chart, my assumption appears to be justified as both the graphs for the reduced Norway and the Albino are found to run a similar course This relation is acceptable, since, as shown in the tables given by Donaldson and Hatai ('11), and also by Miller ('11), the relative weight of the brain in the mature albino rat is 12 to 16 per cent less than in the Norway rat of like body weight, and, furthermore, the relative weight of the

body in the Albino is about 20 to 40 per cent less than in the Norway rat of like age (table 9) Accordingly, the albino brain should be about 18 per cent or more less than the Norway brain of like age, and the data for the thickness of the cortex in the two forms show a fairly constant relation, when plotted as in chart 9 in accordance with this assumption (see also table 3, Sugita, '17 a)

As stated, Norway rats under about 10 days of age have not been studied, but a comparison of the graph for the thickness of the cortex in the normal albino rat with the graph for the Norway cortex displaced for age makes it reasonable to assume that a Norway brain which weighs 1 16 grams (Group N XI) corresponds to an albino brain which weighs about 0 95 grams (Group IX), at which stage the cerebral cortices of the both forms have nearly completed their active growth in thickness and are going over to the second phase, during which the cortical area keeps pace with the increase in brain volume It may be assumed also (see later) that, in the Norway rat, with a brain weight of about 1 4 grams the cortical myelination is beginning to take place

Thus in the postnatal life of the Norway rat, the first phase of the development of the cerebral cortex covers the period during which the brain weight increases to 1 16 grams from birth when the brain weight is about 0 25 grams, and the second phase of the cortical development covers the period, during which the brain weight increases from 1 16 grams to about 1 41 (Group N XIV) when the cortex attains within 4 per cent the full thickness By the middle of the second phase the process of myelination is active, and before the end of this phase the cortex has already attained nearly its full thickness

This assumption that the completion of the cortical development in thickness coincides with the period of active myelination, is supported by another set of facts Table 10 gives the absolute weights of the dry substance in the brain of the Norway rat arranged according to brain weight These values were calculated by me from tables originally given by Donaldson and Hatai (11) The data are plotted in chart 10 which also gives the corresponding data for the albino rat in a dotted curve

TABLE 10

*Giving the weight of the dry substances in the brain of the Norway rat according to brain weight Based on the observed data given by Donaldson and Hatai ('11), in p 448, Jour Comp Neur , vol 21 Both sexes averaged *Males only*

TOTAL BRAIN WEIGHT	WEIGHT OF THE DRY SUBSTANCES IN THE BRAIN	TOTAL BRAIN WEIGHT	WEIGHT OF THE DRY SUBSTANCES IN THE BRAIN
grams	*grams*	*grams*	*grams*
0 25	0 041	1 55	0 309
0 35		1 65	0 339
0 45		1 75	0 377
0 55		1 85	0 400
0 65	0 067*	1 95	0 407
0 75	0 100	2 05	0 445
0 85	0 100	2 15	0 460
0 95		2 25	0 498
1 05		2 35	0 500
1 15	0 155	2 45	0 540
1 25	0 210	2 55	0 534
1 35	0 229	2 65	0 575
1 45	0 291	2 75	0 600*

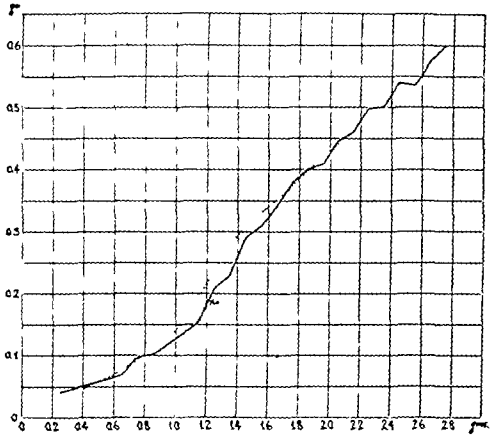

Chart 10 Giving the absolute weights of the dry substance in the Norway brain, arranged according to brain weight, based on the observations of Donaldson and Hatai ('11), accompanied by the corresponding data for the albino rat, in a dotted line × and * show the turning points of the curves

This chart shows clearly that the solids in the Norway brain increase rapidly after the brain weight has reached something more than 1 2 grams (see ×) This turning point of the graph corresponds to 0 95 grams of brain weight in the Albino (see *) It was found in the albino rat that, when the brain weight has surpassed 1 15 grams, namely 0 95 plus 0 20 grams, myelination of the cortical fibers is active Hence, in the Norway brain, the myelination in the cortex should be active when the brain weight has reached 1 44 grams, namely somewhat more than 1 2 plus 0 2 grams Furthermore, as we have seen that in the albino rat the beginning of myelination in the cortex coincides with the phase when the cortex has nearly attained its full thickness, so we see the same relations in the Norway rat also

From these facts we conclude that the brains of the both forms pass through the same course of cortical development according to age, as the span of life is the same in the two The weights of the brains which are in the same stage of development, are however not the same in the both forms, being in the Norway rat about 18 per cent—the Norway brain weight being taken as the standard—heavier than in the albino rat The statement of Donaldson which was expressed in the paper cited to wit If in the animals compared the brain weights are the same then the Norway rat has a smaller body weight and a higher percentage of water in the central nervous system ' might be rewritten as follows When ages are the same, the Norway rat has a greater body weight, a heavier brain (18 per cent more in weight) a thicker cortex and nearly the same percentage of water in the central nervous system

A comparison of the cortical development in the two forms can be made adequately only by first reducing by 18 per cent the actual brain weight of the Norway rat and then comparing the cortex in both forms according to the corrected brain weight Since mature Norway brains have only a slightly greater volume than the Albino brains of like weight (see table 4 A, Sugita 18) but at the same time have a cerebral cortex on the average 8 per cent thicker, it follows that in the Norway brain the proportion of gray substance is greater This difference apparently accounts for the higher percentage of water found in the Norway brain

VIII SUMMARY

1 The thickness of the cerebral cortex of the Norway rat has been systematically investigated, employing as material 36 males and 18 females, all over 17 grams in body weight, and using uniformly the methods which were adopted by me for the investigation of the cerebral cortex of the albino rat

2 The observed data are first given and later are corrected to the values for the fresh condition of the material The corrected data are given fully in tables and in charts

3 The relations of the cortical thicknesses at the several localities measured are quite similar among themselves to those found in the albino rat The average thickness of the cortex in the adult Norway rat is always higher (1 to 28 per cent) than that of the corresponding locality in the adult albino rat The occipital cortex is better developed (thicker) in the Norway rat This is to be associated with the more perfect visual apparatus in the Norway rat

4 As to the phases of development of the cortical thickness, a Norway brain of a given age corresponds to an albino brain, which weighs about 16 to 20 per cent less The Norway brain weighing 0 25 to 1 16 grams (Groups N II to N XI) is in its first phase of active development which corresponds to an Albino brain weighing 0 25 to 0 95 grams The Norway brain weighing 1 16 to 1 44 grams (Groups N XI to N XIV) is in its second phase of development of the cortex corresponding to the albino brain weighing 0 95 to 1 15 grams

5 The cortex of the Norway rat attains nearly its full thickness at the time when the brain weighs somewhat more than 1 44 grams, corresponding to the age of twenty days and to a body weight of something more than 36 grams At this phase probably the rapid myelination of the fibers in the cerebral cortex is taking place

6 The general average thickness of the cortex in the mature Norway rat is 2 06 mm , exceeding by about 8 per cent that of the albino rat brain of the same weight

7 Owing to the greater thickness of the cerebral cortex the mature Norway brain contains more gray matter than does the albino brain of like weight and this excess of gray matter explains the somewhat higher percentage of water found in the Norway brain

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METABOLIC ACTIVITY OF THE NERVOUS SYSTEM

II. THE PARTITION OF NON-PROTEIN NITROGEN IN THE BRAIN OF THE GRAY SNAPPER (NEOMAENIS GRISEUS) AND ALSO THE BRAIN WEIGHT IN RELATION TO THE BODY LENGTH OF THIS FISH

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ONE CHART

The prime object of the present investigation was to extend some observations made recently concerning the metabolic activity of the central nervous system of the albino rat (Hatai, '17) to the nervous system of lower vertebrates. It was my hope that such a comparative study might yield valuable data for an understanding of the complex phenomena of metabolism in this important organ.

In the course of the present investigation I was able to accumulate a considerable amount of data on the weight of the brain together with its water content, a study which has revealed several interesting facts which have not been yet fully appreciated, so that I have decided to present these data also in the following pages. In connection with this work, it is a pleasure to acknowledge my indebtedness to Dr. A. G. Mayer, Director of the Department of Marine Biology of the Carnegie Institution of Washington. Dr. Mayer not only granted me the privileges of the laboratory at the Dry Tortugas, but gave me encouragement and many helpful suggestions throughout the course of this work.

contrasted with the observed values The agreement is highly satisfactory, and thus the formula may be employed when the probable brain weight of the gray snapper in which body length is known, is desired I may point out that the absolute amount of increment of the weight of the brain following every millimeter increase of the body length is slightly over four milligrams (4 33 milligrams)

PERCENTAGE OF WATER IN THE BRAIN

Altogether 64 snappers were examined to determine the water content in the brain, and the results have been already given n table 1 An examination of the table reveals several striking relations in regard to the percentage of water The percentage of water given by the smallest fish is 78 85 per cent while that of the larger fish, having a body length of 424 mm and ranking in length third from the largest in which the water determination was made, gives 78 54 per cent The frequency distribution of the percentage of water gives the following results

TABLE 3

Showing the frequency distribution of the percentage of water in the brain of the gray snapper

PER CENT OF WATER	NUMBER OF CASES
75–76	2
76–77	5
77–78	15
78–79	17
79–80	16
80–81	6
81–82	2
82–83	1
Total number	64

Despite the fact of a wide range in the percentage o water, the distribution of the frequencies is practically normal, and furthermore the high and low values are well mingled, when these

values are arranged according to the body length of the snapper (table 1) and there is no noticeable tendency for the lower values of the percentage of water to occur more frequently among larger fish, or vice versâ. From this we infer that so far as the present data are concerned, the percentage of water in the small and large fish is nearly identical within a wide range of body length, and therefore the percentage of water does not vary regularly with the length or size of the fish. The average of 64 determinations gives the percentage of water as 78.61 per cent.

This wide variation in the percentage of water I am unable to explain at the present moment. It was thought at first that the method of capture, particularly the use of dynamite might be responsible for it. Careful examination however (see remarks in table 1) of the table shows at once that such is not the case, and these wide variations are not correlated with the method of capture. It is true that the cranial cavity of the fish contains liquid as well as a jellylike substance, and the adhesion of particles of this substance may alter to some extent the percentage of water, but this factor is too insignificant to cause the wide variations shown in the table.

One other factor, though it appears to be important, cannot be readily tested, namely, masked age; that is a failure of the size and weight of the fish to indicate the age. We have no way to determine the age of the gray snapper. It may be that the size of the fish shows a wide range of variation for any given age. If size was positively correlated with age, then the low percentage of water would be given by the older fish, and vice versâ. Therefore should we be able to arrange the data according to the ages of the fish, not the size of the fish as has been done, the values for the water should arrange themselves in a regular descending order with increasing age. This is, however, a mere speculation and must wait the test of future investigation.

Still another possible factor is the low grade of organization of the fish brain compared with that of the higher vertebrates. It is conceivable that owing to this low grade of organization, the structural maturity, or especially the process of myelination, may not progress regularly, and that within the same size or at

MATERIAL USED

The gray snapper, Neomaenis griseus, was chosen for this investigation not only because these fish are abundant in subtropical seas, but also because they possess numerous virtues for experimental purposes The snapper may be kept in the laboratory for a long period, and in captivity as well as when free, takes almost any kind of food, cooked or raw, animal or vegetable The fish is well known for sagacity and boldness and is suited for various kinds of experimentation Indeed the snapper has already been carefully studied by Reighard ('08) as to its behavior Thus, with the hope that the gray snapper may in future prove to be a suitable form for certain lines of experimental work, I have utilized all the brains which have been used for chemical investigation, together with some others, for studying the growth of the brain in weight with respect to body length Most of the fish were secured by netting them, but on account of the difficulty of getting the larger fish by this method, I have also used dynamite as well as the hook and line I have noted in table 1 the method adopted for catching each individual

TECHINQUE EMPLOYED

The fish were examined as soon as they were brought into the laboratory However, as in the case of netting them, when too many were caught at once some were kept in a live box for not more than two days, except in a few cases in which they were kept for special purposes for several days When the fish were kept in a live box for more than two days it is so stated in table 1

In every instance the length of body was recorded in the following way The fish was laid on its side and the length was determined by means of calipers from the tip of the snout to the middle of the caudal edge of the tail The body weights of the fish were also taken in a few instances Although I realized the desirability of recording the body weight in all cases, yet it was not always possible to make this measurement

TABLE 1

Showing the brain weights according to various body lengths together with the percentage of water in the brain of the gray snapper Arranged according to increasing body length

BODY		BRAIN WEIGHT	WATER IN BRAIN	REMARKS
Length	Weight			
mm	*grams*	*grams*	*per cent*	
88	12	0 122	78 80	Net
137	43	0 234	78 63	Net
150	59	0 284	79 33	Net
215		0 622	79 52	Net
216	140	0 628	81 12	Dynamited
217		0 483	78 31	Net
220		0 670	79 00	Net
227	173	0 627	80 43	Dynamited
237		0 675	78 19	Net
238	197	0 660	79 01	Hook
240		0 711	79 02	Net
245	220	0 732	81 48	Dynamited
249	218	0 723	80 69	Dynamited
252		0 748	79 00	Net
252		0 762	78 61	Net
253	229	0 897	80 65	Dynamited
256		0 748	77 51	Net
258		0 828	79 47	Net
259		0 844	78 32	Net
262		0 833	78 75	Net
262		0 882	77 80	Net
263		0 864	77 29 ♀	Net
263	269	0 803	80 08	Hook
263	261	0 816	79 68	Hook
268		0 859	79 71	Net
269		0 781	77 49	Net
271		0 921	78 78 ♂	Net
277		0 816	78 57	Net
278		0 843	78 32	Net
278	311	0 871	82 91	Hook
285		0 985	79 17 ♀	Net
293		1 000	77 28 ♂	Net
294		0 861	78 86 ♂	Net
295		0 925	77 56 ♂	Dynamited
296		0 900	78 07	Net
298		0 982	78 49 ♂	Kept in live box 4 days
300		0 907	79 21 ♂	Dynamited
300		0 971	78 17 ♀	Net
301		0 952	77 10 ♂	Net

TABLE 1—Continued

BODY		BRAIN WEIGHT	WATER IN BRAIN	REMARKS
Length	Weight			
mm	*grams*	*grams*	*per cent*	
302		1 042	77 87 ♀	Net
302		1 042	77 06 ♂	Net
303		0 776	75 40 ♀	Dynamited
306		1 079	79 17 ♀	Kept in live box
317		0 974	78 03 ♂	Net
318		1 072	79 57 ♀	Net
330		1 124	76 51 ♂	Net
335	681	1 164	80 07 ♀	Kept several days in box
336		1 124	77 67 ♀	Kept several days in box
340	908	1 126	76 16 ♀	Net
345		1 061	79 15 ♀	Net
348	1¹ lbs	1 141	77 46 ♀	Net
353	2 lbs	1 117	76 63 ♀	Net
353	2 lbs	1 169	77 81 ♀	Net
360		1 178	77 88 ♂	Net
362		1 257	78 65 ♂	Net
367	781	1 262	76 67 ♂	Hook
369		1 286	77 08 ♀	Net
374	2 lbs	1 249	76 24 ♂	Net
380	3 lbs	1 281	75 33 ♂	Net
385		1 418	♂	Net
390		1 336	77 59 ♂	Net
392		1 353	79 67 ♂	Net
392		1 644	♀	Dynamited
401		1 584	♀	Dynamited
408		1 618	♀	Dynamited
416		1 632	♂	Dynamited
424		1 369	78 54 ♀	Dynamited
430		1 400	♀	Dynamited
432		1 424	79 97 ♂	Dynamited
438		1 530	♂	Dynamited
439		1 400	♀	Dynamited
439		1 499	80 00 ♀	Dynamited
441		1 601	♀	Dynamited
448		1 591	♀	Dynamited

As soon as the brain was exposed by means of a small bone forceps, it was separated from the spinal cord between the first vertebra and the base of the skull It was not practicable to find the first spinal nerve or to determine the caudal end of the fourth ventricle, methods which are usually adopted in separat-

ing the brain from the spinal cord in the mammalian nervous system Anteriorly the olfactory nerves were cut close to the olfactory bulbs The saccus vasculosus was not included with the brain The brain which was thus removed was placed in a small bottle which had been previously weighed, and this was weighed again to a milligram After the fresh weight of the brain was determined, the bottle with its contents was placed in a steam oven at a temperature of 80°–90°C for several days (Tortugas laboratory) and then later dried at the Wistar Institute under better laboratory conditions at 96°C The various other methods used for the analysis of the brain will be described later

THE BRAIN WEIGHT IN RELATION TO BODY LENGTH

Altogether observations on 74 brains of the gray snapper have been made

From table 1 the average brain weight of the snapper for several values of the body length has been calculated and the results are given in table 2

In order to show the general distribution of the brain weights in relation to the body length, I have prepared a chart based on the data given in tables 1 and 2

In the chart males and females are not distinguished As will be seen from chart 1 the distribution of brain weight in respect

TABLE 2

Showing the average brain weight of the gray snapper for the several values of the body length

BODY LENGTH RANGE	BODY LENGTH OBSERVED	BRAIN WEIGHT		NUMBER OF BRAINS
		Observed	Calculated by formula	
mm	*mm*			
200–250	231	0 643	0 667	10
250–300	271	0 860	0 840	23
300–350	319	1 037	1 048	15
350–400	373	1 296	1 282	12
400–450	428	1 513	1 500	11
Average		1 070	1 071	

to the increasing body length from 150 mm upward is practically linear This linear relation between these two characters is better shown by the positions of the averaged values, which are also plotted It is well known that in the adult stage the relation between brain weight and body length or body weight is practically linear, even in the case of some mammals (see for instance growth of brain in weight in the albino rat in respect to body length or body weight, Donaldson, '09) but it is remarkable to find the linear relation in fish when they are so small This linearity during the period of early growth probably means that in the gray snapper the brain reaches its struc-

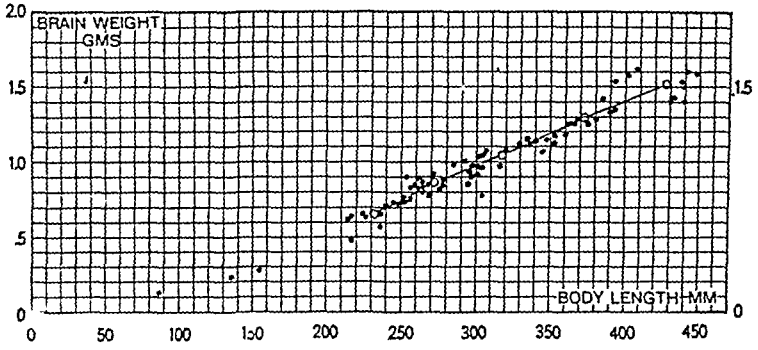

Chart 1 Showing the weight of the brain of the gray snapper according to body length The observed weights are represented by 74 fish • = observed weight — o — o — = average observed weight (table 2)

tural maturity early, and that the subsequent increase in weight indicates merely a uniform swelling of the nervous system as a whole The maturity of the brain at a relatively early stage of growth may be inferred also from practical constancy of the percentage of water in the brain from the very small to the very large fish in this series (page 48)

It is to be regretted that it was not possible to obtain data on smaller specimens, though every effort was made to obtain such specimens while I was at the Tortugas Laboratory We were even unable to find any of the gray snapper fry, though the fry of the school master (Neomaenis apodus) which is most closely

related to the gray snapper, was abundant everywhere Possibly the months of June and July were not a proper season to find them, or the fry of the gray snapper may not live in the open seas or along the beach, but may be in hiding under the intricate roots of mangroves, a tree not found on the Tortugas Islands

On account of the scantiness of the data on the gray snapper less than 200 mm in body length, I am unable to present a complete record of the growth of the brain However it appears from the general trend of the growth curve that, with the possible exception of the very early period, the relation between the brain weight and body length does not deviate much from linearity

Kellicott ('08) who studied the growth of the brain in the smooth dogfish (Mustelus canis, Mitchill) in respect to the body weight, found the graph to resemble that for the mammalian brain, that is the graph shows a rapid rise at the early period which is followed by a slower rate of growth The form of the curve suggests a logarithmic formula such as was used to represent the growth of the brain in the albino rat (Hatai '09) In other words the form of the graph for the gray snapper is strikingly different from that for the dogfish This difference may be due to the fact that in the dogfish the brain possesses a voluminous cerebellum as well as olfactory bulbs, and the combined weights of these two structures may be greater than that of the rest of the brain while these two structures in the gray snapper are very small and the latter was not included It appears that these two parts olfactory bulbs and cerebellum, of the dogfish brain grow very rapidly during the earlier period thus giving the form of the graph similar to that for the mammal

Since the brain weight of the gray snapper shows a linear relation to the body length through a wide range and since the fish which are usually caught fall within this range I have devised the following formula for brain weight on body length in hopes that it may prove useful for some future investigation

Brain weight (gms.) = 0.00133 Body length (mm.) − 0.333

The results of the calculation are given in table 2 and there

contrasted with the observed values The agreement is highly satisfactory and thus the formula may be employed when the probable brain weight of the gray snapper in which body length is known, is desired I may point out that the absolute amount of increment of the weight of the brain following every millimeter increase of the body length is slightly over four milligrams (4 33 milligrams)

PERCENTAGE OF WATER IN THE BRAIN

Altogether 64 snappers were examined to determine the water content in the brain, and the results have been already given in table 1 An examination of the table reveals several striking relations in regard to the percentage of water The percentage of water given by the smallest fish is 78 85 per cent while that of the larger fish, having a body length of 424 mm and ranking in length third from the largest in which the water determination was made, gives 78 54 per cent The frequency distribution of the percentage of water gives the following results

TABLE 3

Showing the frequency distribution of the percentage of water in the brain of the gray snapper

PER CENT OF WATER	NUMBER OF CASES
75–76	2
76–77	5
77–78	15
78–79	17
79–80	16
80–81	6
81–82	2
82–83	1
Total number	64

Despite the fact of a wide range in the percentage o water, the distribution of the frequencies is practically normal, and furthermore the high and low values are well mingled, when these

values are arranged according to the body length of the snapper (table 1) and there is no noticeable tendency for the lower values of the percentage of water to occur more frequently among larger fish, or vice versâ From this we infer that so far as the present data are concerned, the percentage of water in the small and large fish is nearly identical within a wide range of body length, and therefore the percentage of water does not vary regularly with the length or size of the fish The average of 64 determinations gives the percentage of water as 78 61 per cent

This wide variation in the percentage of water I am unable to explain at the present moment It was thought at first that the method of capture, particularly the use of dynamite, might be responsible for it Careful examination however (see remarks in table 1) of the table shows at once that such is not the case, and these wide variations are not correlated with the method of capture It is true that the cranial cavity of the fish contains liquid as well as a jelly like substance, and the adhesion of particles of this substance may alter to some extent the percentage of water, but this factor is too insignificant to cause the wide variations shown in the table

One other factor though it appears to be important, cannot be readily tested, namely, masked age, that is a failure of the size and weight of the fish to indicate the age We have no way to determine the age of the gray snapper It may be that the size of the fish shows a wide range of variation for any given age If size was positively correlated with age, then the low percentage of water would be given by the older fish and vice versâ Therefore should we be able to arrange the data according to the ages of the fish, not the size of the fish as has been done the values for the water should arrange themselves in a regular descending order with increasing age This is however, a mere speculation and must wait the test of future investigation

Still another possible factor is the low grade of organization of the fish brain compared with that of the higher vertebrates It is conceivable that owing to this low grade of organization, the structural maturity, or especially the process of myelination, may not progress regularly, and that within the same size or at

the same age, a wide range of variation might exist in respect to the degree of myelination, according to the environment of the fish or to the general nutritional conditions Whether or not this suggestion has a value, only further investigation can determine

Scott ('12) found that the percentage of water in the brain of the smooth dogfish differs very little between small and large specimens, and gives on the average 78 5 per cent Donaldson ('05) who examined the brains of the summer flounder (Paralichthys dentatus) noted also but slight variation in the percentage of water in the brains of large and small individuals The average from sixteen flounders in which the body weight ranges from 539 grams to 1290 grams, is 78 45 per cent Thus the average percentages of water obtained by Donaldson, Scott and by myself are 78 45 per cent (flounder), 78 5 per cent (dogfish) and 78 61 per cent (gray snapper) respectively For the purpose of comparison I gave the percentages of water in the brain of several fish, as determined by various investigators

As will be seen from table 4 despite the widely different sizes and probably wide differences in the age of fish, the percentages of water in the brains are very close to one another, and further interest lies in the fact that the values given by the fish brains are not much different from the percentage of water in the adult mammalian brain

Since the reduction in the water in the brain is induced by the deposition of the so called 'myelin substance' (Donaldson, '16) we may infer that the process of myelination in the fish brain attains its mature form at a very early period[1] thus permitting but very slight variation from small to large individuals Scott ('12) also concludes from his observations on the water content

[1] In a private communication Dr G W Bartelmez informs me that in Ameiurus melas, larvae 10 to 12 mm long show already well advanced myelination of the roots of all the cranial nerves, as well as of the fasciculus longitudinalis medialis The age of the larvae, according to Dr Bartelmez's estimate, is about ten to twelve days after fertilization The largest adults measure as much as 120 mm or nearly ten times the length of the larvae in which the myelination is already well advanced From the above we may safely assume that myelination takes place in the fish at an early stage of development

of the dogfish that the differences in the reduction of water in the two cases is that "the nervous (and body) changes which occur in the mammal are post-embryonic and extra utero In the

TABLE 4

Showing the percentage of water in the brain of several fish Data compiled from various sources

SPECIES	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	PERCENT OF WATER	LIPOID-ETHER EXTRACT	SEX	OBSERVER
Cyprinus carpio				77 50	8 33		Von Bibra (1854)
Cyprinus barbus				78 00	9 37		Von Bibra (1854)
Salmo farco				78 90	8 42		Von Bibra (1854)
				80 00			
Lucius esox				81 93	7 25		Von Bibra (1854)
Fish					9 10		Schlossberger (1856)
Cyprinus auratus				77 80			Bezold (1857)
Summer flounder	530	393	0 253	78 05			Donaldson (1905)
Summer flounder	540	397	0 305	79 06		♂	Donald on (1905)
Summer flounder	540	386	0 351	78 00		♂	Donald on (1905)
Summer flounder	560	411	0 338	78 70			Donald on (1905)
Summer flounder	630	409	0 270	78 06		♂	Donaldson (1905)
Summer flounder	640	404	0 293	78 43		♀	Donaldson (1905)
Summer flounder	680	405	0 288	79 56			Donald on (1905)
Summer flounder	834	440	0 311	78 60		♀	Donaldson (1905)
Summer flounder	840	467	0 358	78 08		♀	Donald on (1905)
Summer flounder	860	453	0 406	78 37		♀	Donaldson (1905)
Summer flounder	880	459	0 381	77 11		♂	Donaldson (1905)
Summer flounder	890	459	0 417	77 98		♀	Donaldson (1905)
Summer flounder	1010	460	0 350	78 22		♀	Donald on (1905)
Summer flounder	1010	447	0 369	78 72		♂	Donaldson (1905)
Summer flounder	1080	478	0 412	79 06		♀	Donaldson (1905)
Summer flounder	1290	503	0 391	78 27		♀	Donaldson (1905)
Average				78 45			
Mustelus canis[1]				78 5			Scott (1912)
Barracuda	12 lbs	104	1 554	79 39		♂	Hatai (1917)
Neomaenis griseus[2]				78 61			Hatai (1917)
Cherna americana							
Red Grouper	14½ lbs	80	1 230	78 80			Hatai (1917)
Shark Sp?	160 lbs		3 093	80 07		♂	Hatai (1917)

[1] Average of 97 determinations from very small to very large Percentage of water shows very slight variation

[2] Average of 51 gray snappers Range of variation is shown in table 11

dogfish they take place in utero" He, however, has not determined the water content in the brain of the dogfish in utero

From the foregoing it is clearly important to determine the water content in the brain of the fish at very early stages in order to discover the period of rapid reduction which must take place in consequence of the appearance of myelin in the brain It is the hope of the writer to do this in the near future

CHEMICAL ANALYSIS OF THE BRAIN (GRAY SNAPPER)

Utilizing the materials which were employed for the determination of the percentage of water, I have determined the nitrogen in the total solids, as well as the amount in the ether-alcohol soluble fraction extracted from the total solids The results of these determinations are shown in table 5

TABLE 5

Showing the amount of the ether-alcohol soluble and insoluble fractions in the brain of the gray snapper also the amount of nitrogen in the total solids, as well as the nitrogen in the ether alcohol fraction

SERIES	BRAINS		SOLIDS	WATER	TOTAL NITROGEN	WEIGHT OF		NITROGEN IN	
	NUMBER	FRESH				Residue	Alcohol ether Extract	Residue	Alcohol ether Extract
		weight		*per cent*	*mgms*	*gms*	*gms*	*mgms*	*mgms*
1	28	27 303	5 665	79 14	462	2 707	2 958	364	98
					8 15%	47 79%	52 21%	78 79%	21 21%
2	19	20 013	4 588	77 07	334	1 938	2 650	269	65
					7 28%	42 24%	57 75%	80 54%	19 46%
Average				78 11	7 72%	45 02%	54 98%	79 67%	20 33%

To carry out the determinations presented in table 5, I have divided the entire materials into two groups in which group 1 gives for the brain a percentage of water which ranges between 78 per cent and 80 per cent, while in group 2 the percentage of water ranges between 76 per cent and 77 per cent All the other brains which gave percentages of water beyond these limits were excluded Since all these data for the fish may be discussed conveniently by comparing them with similar data obtained

from the rat brain, I may state simply that the values for the alcohol ether soluble fraction obtained in this series of fish are similar to those obtained by Von Bibra ('54) and by Schlossberger ('56) in other forms of fish (table 3)

CONTENT OF NON PROTEIN NITROGEN IN THE BRAIN

Altogether 44 snappers of medium size were used for the purpose of determining the various extractive nitrogenous substances in the brain These brains were divided into three samples each giving nearly the same amount of moist brain weight One additional sample was obtained from the brains of the schoolmaster (Neomaenis apodus) which is a species most closely related to the gray snapper

The fresh brains of each sample were ground finely and then preserved in 150 cc of 2 5 per cent solution of trichloracetic acid in water The ground brains were transferred to a bottle by means of 50 cc water, thus making altogether 200 cc of solution The filtrates from this mixture were brought back to the Wistar Institute for analysis The methods used for the determination of various nitrogen fractions were as follows

1 Total non protein nitrogen Micro method of Folin and Farmer as modified by Benedict and Bock

2 Amino acid nitrogen Van Slyke's nitrous acid method Also the same author's micro apparatus

3 Urea nitrogen Urease method

4 Ammonia nitrogen By the usual aeration method

In all cases except the case of the amino acid the nitrogen content was determined by means of the DuBosecq colorimeter The results obtained from these determinations are given in table 6

Since it is my intention to discuss this subject later in comparison with the similar data recently obtained from the brain of the albino rat I shall merely direct attention to the fact that these three samples give results very close to each other Furthermore the results obtained from the sample of the schoolmaster also agree with those found in the case of the gray snapper

TABLE 6

Showing nitrogen content in terms of the non-proteins, the amino acids, the urea and the ammonia, in the brains of the gray snapper and of the 'schoolmaster'

SERIES	BRAINS		MILLIGRAMS NITROGEN PER 100 GRAMS OF FRESH BRAIN				
	Number	Weight	Non Protein	Amino acids	Urea	Ammonia	Undetermined nitrogen
			Neomaenis griseus				
		gms					
1	16	13 166	204	101 8	13 2	17 7	71 3
2	13	10 713	224	125 0	17 8	18 9	62 3
3	15	12 048	203	121 2	15 8	17 4	48 6
Average		11 976	210	116 0	15 6	18 0	60 7
			Neomaenis apodus				
1	10	11 195	225	126 0	17 3	17 2	64 5

This agreement in the various substances might also be taken to support the belief of the systematists that these two species are closely related

COMPARISON BETWEEN THE GRAY SNAPPER AND THE ALBINO RAT IN REGARD TO THE CHEMICAL COMPOSITION OF THE BRAIN

In order to compare the data on the chemical composition of the brain in the gray snapper with those for the brain of the albino rat, table 7 was prepared The entries for the fish are based on tables 5 and 6, while the data on the albino rat were obtained from an earlier paper (Hatai, '17)

When comparison is made between the fish brain and the entire brain of the albino rat, we find a distinct difference in regard to the content of the total nitrogen and of the nitrogen in the lipoids, as well as in the total amount of the ether-alcohol extractive materials These differences must undoubtedly be correlated with anatomical differences in the two forms of the brains In the rat we find a well developed cerebrum and cerebellum in which the myelinated nerve fibers are relatively less than in the stem, while the cell bodies are more abundant On the other hand in these fish brains we find a mere trace of the

TABLE 7

Showing the comparison of the gray snapper with the albino rat in regard to the chemical composition of their brains

	GRAY SNAPPER ENTIRE BRAIN	ALBINO RAT STEM OF ENCEPHALON	ALBINO RAT ENTIRE BRAIN
Water in brain per cent	78 11	76 16	77 96
Total nitrogen in fresh tissue per cent	1 69	1 89	1 95
Total nitrogen in solids per cent	7 72	7 76	8 98
Alcohol-ether extract in solids per cent	54 98	55 03	47 14
Nitrogen in alcohol-ether soluble fraction per cent	20 60	19 90	18 20
Percentage of water in lipoid free tissue per cent	88 80	87 06	87 00
Milligrams of non protein nitrogen per 100 grams of fresh tissue milligrams	220	150	159
Partition of nitrogen in milligrams of nitrogen per gram of solids			
Non protein N	0 6	0 0	7 6
Amino acids N	0 3	2 9	3 5
Urea N	0 7	0 7	0 7
Ammonia N	0 8	0 6	0 7
Partition of non protein nitrogen in percent of protein nitrogen			
Non proteins	13 04	9 72	10 37
Amino acids	7 20	4 68	4 60
Urea	0 97	1 05	0 95
Ammonia	1 11	1 01	1 01

cerebrum and cerebellum compared with the size of the stem in which the myelinated nerve fibers are abundant Consequently we should expect a higher value of the total nitrogen in the rat brain than in the fish brain, since the former possesses relatively a much greater number of cell bodies in those two well developed parts, the cerebrum and cerebellum At the same time the rat brain ought to give relatively a less amount of lipoids, owing to the greater abundance of the gray matter in the predominant parts In the fish brain the insignificant growth of the cerebrum and cerebellum makes the stem of the brain relatively predominant in the quantitative relations, and since the stem is the portion of the brain in which the myelinated fibers are mostly found, we should expect the percentage value of the lipoid fraction in the fish brain to be relatively higher than in the rat

If we compare now the entire brain of the snapper with the stem of the albino rat brain (table 7) we notice a surprisingly close similarity This we should expect since as was already stated the fish brain is practically represented by the stem, since the cerebral and cerebellar portions are relatively insignificant Thus we notice the practical identity in the percentage values of the total nitrogen, lipoid nitrogen, and the amount of the lipoids The percentage of water in the stem of the rat is however far less than in the entire brain of the fish which may be accounted for by the fact that in the brain of the fish the cerebrum and the cerebellum, though small in relative quantity, nevertheless are composed of structures rich in water, and thus bring the value of the water higher in the fish than in the stem alone of the albino rat brain

The nitrogen content of the lipoid is slightly higher in the fish brain than in the albino rat brain, though almost identical with that in the stem This difference may be due to the quantitative difference in the proportion of various lipoids in which the nitrogen content is not the same

I now wish to consider the partition of the non-protein nitrogen in the fish brain compared with the brain of the albino rat As will be seen from table 7 the content of the non-protein nitrogen is considerably greater in the fish than in the rat brain We also notice that the greater part of the non-protein nitrogen is represented by the nitrogen of the amino acids The nitrogen values given by both the urea and ammonia are small and are practically identical both in the fish and rat The greater amount of non-protein nitrogen found in the fish brain in comparison to the rat is interesting, though I am unable to explain this difference satisfactorily I wish however to call attention to two factors which may have some bearing on the difference just noted

1 It seems probable that on account of the low grade of organization of the fish brain the physical consistence of the nervous system may not be as stable as that of the more highly organized mammalian nervous system, and thus the wear and tear process may be greater and produce a correspondingly greater amount of waste products in the fish brain

2 According to Folin and Denis ('14) the normal human blood contains, on the average of four cases, 32 milligrams of non-protein nitrogen per 100 cc of blood, while Wilson and Adolph ('17) found in the blood of various fresh water fish much higher values for the non-protein nitrogen (42 mgms per 100 cc) than in the human blood, and furthermore these investigators found a greater fraction of the non-protein nitrogen was represented by the nitrogen of amino acids (23 mgms per 100 cc or about 55 per cent of the total non protein nitrogen) Thus my own observations on the fish brain closely agree with those of Wilson and Adolph on the fish blood, so far as the relative abundance of the non-protein nitrogen is concerned, as well as in the relation of the amino acid nitrogen to the total non-protein nitrogen

Denis ('13–'14) found also a considerably greater amount of non protein nitrogen in the blood of marine fishes when contrasted with human blood Denis found 62 mgms of non protein nitrogen per 100 cc of blood (average of 10 species of teleosts) and as high as 1087 mgms in the case of the elasmobranchs (average of three species) Thus the greater abundance of the non-protein nitrogen in the fish blood, accompanied by a slow circulation, might be largely responsible for a greater accumulation of the non-protein nitrogenous extractive substances in the fish brain

SUMMARY

The gray snapper, Neomaenis griseus, was mainly used for the present investigation The following are the more important facts brought out

1 The relation between brain weight and body length is practically linear This linear relation appears in the fish as small as 150 mm in length The fish smaller than 150 mm were not studied because they could not be obtained

2 The percentage of water in the brain varies very little from small to large (body length 88 mm to 148 mm) A similar relation was observed by Donaldson (05) in the brain of the summer flounder and by Scott ('12) in the brain of the smooth dogfish The probable explanation is that the process of mye